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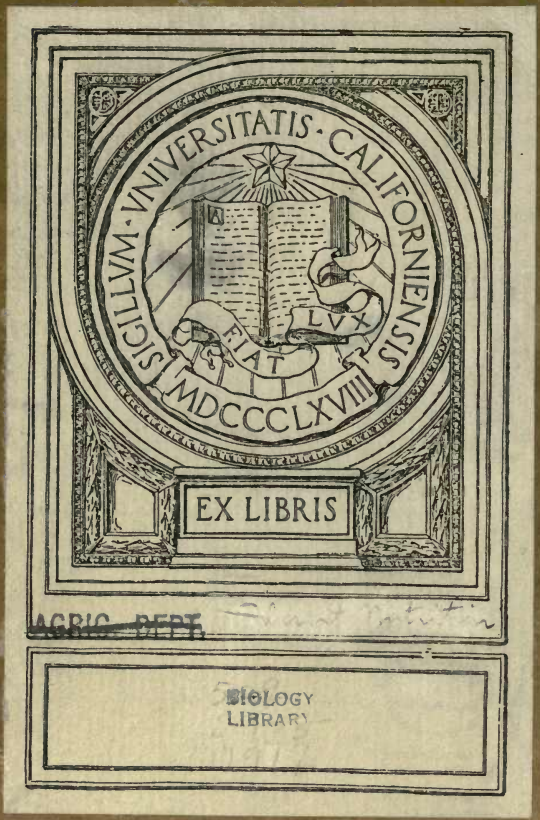

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BY

E. FRANKLAND ARMSTRONG, D.Sc., PH.D., F.I.C.

FELLOW OF THE CITY AND GUILDS OF LONDON INSTITUTE

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GENERAL PREFACE.

THE subject of Physiological Chemistry, or Biochemistry, is enlarging its borders to such an extent at the present time, that no single textbook upon the subject, without being cumbrous, can adequately deal with it as a whole, so as to give both a general and a detailed account of its present position. It is, moreover, difficult in the case of the larger textbooks to keep abreast of so rapidly growing a science by means of new editions, and such volumes are therefore issued when much of their contents has become obsolete.

For this reason an attempt is being made to place this branch of science in a more accessible position by issuing a series of monographs upon the various chapters of the subject, each independent of and yet dependent upon the others, so that from time to time, as new material and the demand therefor necessitate, a new edition of each monograph can be issued without re-issuing the whole series. In this way, both the expenses of publication and the expense to the purchaser will be diminished, and by a moderate outlay it will be possible to obtain a full account of any particular subject as nearly current as possible.

The editors of these monographs have kept two objects in view: firstly, that each author should be himself working at the subject with which he deals; and, secondly, that a *Bibliography*, as complete as possible, should be included, in order to avoid cross references, which are apt to be wrongly cited, and in order that each monograph may yield full and independent information of the work which has been done upon the subject.

It has been decided as a general scheme that the volumes first issued shall deal with the pure chemistry of physiological products and with certain general aspects of the subject. Subsequent monographs will be devoted to such questions as the chemistry of special tissues and particular aspects of metabolism. So the series, if continued, will proceed from Physiological Chemistry to what may be now more properly termed Chemical Physiology. This will depend upon the success which the first series achieves, and upon the divisions of the subject which may be of interest at the time.

R. H. A. P.
F. G. H.

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PREFACE.

TWENTY-EIGHT years ago the late Sir John Burdon Sanderson described one of the aims of Physiology as the acquirement of an exact knowledge of the chemical and physical processes of animal life. The recent history of physiological progress shows that investigations confined to the study of physical and chemical processes have been the most fruitful source of physiological advance, and it is principally the exact chemical study of the substances found in animals and plants which has enabled the physiologist to make this advance.

The last decade has seen very material progress in our knowledge of the carbohydrates, more particularly with regard to their inner structure, biochemical properties, and the mechanism of their metabolism. In consequence, many problems of the greatest fascination for the biochemist have presented themselves for solution.

This monograph aims at giving a summary of the present position of the chemistry of the carbohydrates. The reader is assumed to be already acquainted with the subject so far as it is dealt with in the ordinary textbooks. The available information is, however, so widely scattered in the various scientific periodicals that it is impossible for any one approaching the subject to inform himself rapidly of what has been done. It is to meet such needs that this monograph is primarily intended.

A bibliography is appended, which contains references, classified under appropriate headings, to most of the recent works on the subject and to the more important of the older papers. It makes no claim to be exhaustive but serves to indicate how much is at present being done in this field.

E. F. A.

PREFACE TO THE SECOND EDITION.

Our interest in the carbohydrates has been again aroused by the return of Emil Fischer to the subject. He has announced his acceptance of the γ-oxide formula of glucose which was used in the First Edition of the Monograph to explain all the properties of this carbohydrate. In continuation of his work on the acyl derivatives of glucose he has been able to show the probable composition of the tannins: he seems to think that compounds of this type may be widely distributed in animals and plants and may account for some of the peculiar properties of carbohydrates known to biologists.

It has been found advisable to modify the arrangement of Chapter I. The treatment of the rarer carbohydrates has been extended and, wherever possible, their relation to enzymes has been demonstrated. The chapter on the glucosides has been considerably enlarged and a new chapter, dealing with the significance of the carbohydrates in plant physiology, has been added. The monograph should therefore appeal more generally to those interested in the subject from the botanical and agricultural sides. These problems are some of the most fascinating of those now under investigation, and their study must add to our conceptions of vital change.

It is a pleasant duty to express my thanks to Mr. F. W. Jackson, B.Sc., A.C.G.I., for his help in the revision of the proofs.

E. F. A.

PREFACE TO THE THIRD EDITION.

SINCE the Second Edition of this Monograph was completed the chemistry of the carbohydrates has developed on two main lines, both of which now receive special recognition. The discovery of a third isomeric form of glucose differing from the pentaphane ring forms in structure—probably containing a three-membered (triphane) ring—opens up ways to much future work and in particular has served to elucidate that very vexed problem the structure of sucrose. The discovery of this new glucose derivative also affords an example of the temporary nature of the doctrines of chemical structure : if the arguments for the acceptance of what was known as the γ-oxide formula of glucose had been too rigidly construed there would have been no possibility of a third isomeride. Chemical formulæ are of service so long as they serve to express known facts and stimulate further investigation ; they cease to be of value when used to give expression to observations with which they are not in harmony. We owe the recognition of the new form both to Emil Fischer and to J. C. Irvine, particularly to the latter and his students. The very patient and brilliant work of Irvine on the substituted methyl derivatives of the carbohydrates has done much to increase our knowledge of their structure.

The relationship of optical rotatory power to structure in the case of the carbohydrates has long been a source of speculation, but, because of the indifferent manner in which many of the carbohydrate derivatives had been characterised, nothing definite had been achieved until recently. Owing to the painstaking work of Hudson and his school in America, we are now in possession of many of the necessary data, and the

generalisations of this chemist have given a new and most promising aspect to this field. .

Some of the rarer sugars have been made more available, thus stimulating inquiry ; indeed, as the methods of investigation improve and more attention is paid to the composition of plant products, the occurrence of the scarcer sugars is found to be far more general than had been anticipated, and it may be prophesied that future researches in this direction will be very fruitful. In particular much progress has been made in establishing the structural formulæ of the disaccharides. References to new work have been introduced where appropriate.

Probably in no other branch of chemistry, at all events in that of the aliphatic compounds, is so great an opportunity afforded for the study in detail of the influence of structure on the properties of the molecule. Much has already been done in this direction but we are as yet only on the threshold of the inquiry.

During the past few years most chemists in the allied countries have had to follow more urgent national calls than those of the research laboratory. The British and American nations have, however, learnt to appreciate more fully the need of scientific research, and it is to be expected that in the near future the chemistry of the carbohydrates will be a subject that will attract the attention of workers.

It would have been very difficult for the writer to prepare this edition without the great assistance which he has so freely received from Dr. T. P. Hilditch.

E. F. A.

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INTRODUCTION.

THE carbohydrates, together with the proteins, rank first in importance among organic compounds on account of the part they play, both in plants and animals, as structural elements and in the maintenance of the functional activity of the organism.

The interest attaching to the group may be said to centre around glucose, this carbohydrate being the first to arise in the plant and the unit group from which substances such as cane sugar, maltose, starch and cellulose are derived; it is also of primary importance in animal metabolism, as the main bulk of the carbohydrate in our food materials enters into circulation in the form of glucose.

Under natural conditions the higher carbohydrates are resolved into the simpler by the hydrolytic agency of enzymes, but these also exercise synthetic functions; the simpler carbohydrates are further resolved by processes which are undoubtedly akin to that of ordinary alcoholic fermentation. The carbohydrates are, therefore, of primary importance as furnishing material for the study of the processes of digestion and assimilation.

The carbohydrates are all remarkable on account of their optical characters; it is possible to correlate these with their structure. Of the large number of possible isomeric forms of the gluco-aldohexose, $C_6H_{12}O_6$, sixteen in all, of which glucose is one, only three are met with in nature, although fourteen have already been prepared by artificial means; this natural limitation of the number produced in the plant and utilised by it and by the animal is a fact of great significance and clear proof of the manifestation of a selective process at some period in the evolution of life. The elucidation of these peculiarities invests the inquiry into the nature and functions of the carbohydrates with particular interest and significance.

The simple carbohydrates are all of the empirical composition corresponding with the formula CH_2O, the most important being those containing five or six atoms of carbon. The members of the sugar group are usually distinguished by names having the suffix *ose*.

The simplest carbohydrate, CH_2O, formaldehyde or formal, is in all probability the first product of vital activity in the plant, the carbon

dioxide absorbed from the air being converted into this substance by the combined influence of sunlight and chlorophyll. The conversion of formaldehyde into glucose has been accomplished in the laboratory, but the transformation takes place in such a way that a variety of products is obtained which are optically inactive; there is reason to suppose that but the single substance dextro-glucose is formed in the plant and that this is almost immediately converted into starch; in other words, the vital process is in some way a directed change. The record of the synthetic production of glucose and of the discovery of methods of producing the isomeric hexoses, as well as of determining the structure of the several isomerides, is one of the most fascinating chapters in the history of modern organic chemistry.

A short outline of the ground covered by the complete carbohydrate group may be of value to some readers and will be given before the subject is developed in detail.

The numerical relation existing between the proportions of "carbon" and of "water" in a carbohydrate molecule, $C_m(H_2O)_n$, is the basis of general classification.

The simplest sugars are those in which m and n are equal and range in value from 2 upwards; these are known as *monosaccharides*, and include such carbohydrates as arabinose, $C_5H_{10}O_5$, and glucose, $C_6H_{12}O_6$.

The next, more complex, type of carbohydrate may be regarded, for the moment, as derived either from two molecules of a monosaccharide or from two different monosaccharides by elimination of a molecule of water, and will have the general formula $C_{2m}(H_2O)_{2m-1}$; these are the *disaccharides*, of which cane sugar or sucrose is the most familiar.

Similarly, by elimination of two molecules of water from three of monosaccharide, or of three molecules of water from four of monosaccharide, we arrive at the empirical formulæ for the *tri-* or the *tetra-saccharides*, which are also found in nature.

Important exceptions to this numerical classification are the starches, gums, and celluloses of the general formula $(C_6H_{10}O_5)_x$; these have, of course, little resemblance to the saccharide group beyond their empirical composition; they are of far greater molecular complexity than the sugars, and do not fall within the scope of this work.

The distinctive feature of saccharides other than the monosaccharides is their ready conversion into a mixture of the latter compounds by hydrolytic agency; in other reactions they show the same behaviour as the monosaccharides. The basis of all carbohydrates is

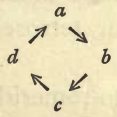
thus the class of monosaccharides, which are systematised according to the number of carbon atoms they contain.

In passing it may be said that the simplest member of the series, CH_2O, formaldehyde, is not a true carbohydrate, since it possesses neither the physical characteristics (a sweet syrup or solid material) nor the chemical (alcoholic) functions of the rest of the sugars.

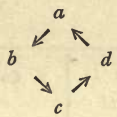
The compound, $CH_2(OH).CHO$, glycollic aldehyde, however, is a sweet-tasting crystalline substance readily soluble in water and possessing all the general properties of the carbohydrates. The next term of the series, $C_3(H_2O)_3$, includes glycerose, $CH_2(OH).CH(OH).CHO$, and dioxyacetone, $CH_2(OH).CO.CH_2(OH)$, and here a further distinction appears, for glycerose is an aldehyde and dioxyacetone a ketone. Moreover, a compound, $CH_3.CH(OH).CH(OH).CHO$, has been prepared to which the name methylglycerose is given; this substance possesses the general properties of a sugar, and analogous compounds such as rhamnose, $CH_3[CH(OH)]_4.CHO$, occur in nature.

The monosaccharides are therefore classified as dioses, trioses, etc., by the number of carbon atoms in the molecule, whilst each class may be subdivided according as it possesses an aldehydic, ketonic, or methyl radicle. So, whilst glucose is an aldohexose, and fructose a ketohexose, rhamnose is a methylaldopentose.

A carbon atom which has four different groups attached to it is known as asymmetric. These groups can obviously be written in order either clockwise:—



or counter clockwise:—



Two different forms of the substance are therefore possible, related as object to image, and they are termed stereoisomerides.

Nearly all the carbohydrates contain asymmetric carbon atoms and display optical activity, and the number of possible stereoisomerides is in many cases very large. Of the monosaccharides the diose, glycollic aldehyde, contains no asymmetric centres and only exists in one form, but in the triose, glyceric aldehyde, there are already possibilities of a dextro- and a lævo-rotatory form, with, of course, the corresponding racemic compound:—

$$\begin{array}{l} CH_2(OH) \\ H.C.OH \\ CHO \end{array} \quad \text{and} \quad \begin{array}{l} CH_2(OH) \\ HO.C.H \\ CHO \end{array}$$

Similarly in the tetroses there are four possible active forms:—

$$\begin{array}{l} CH_2(OH) \\ H.C.OH \\ H.C.OH \\ CHO \end{array} \quad \begin{array}{l} CH_2(OH) \\ H.C.OH \\ HO.C.H \\ CHO \end{array} \quad \begin{array}{l} CH_2(OH) \\ HO.C.H \\ H.C.OH \\ CHO \end{array} \quad \begin{array}{l} CH_2(OH) \\ HO.C.H \\ HO.C.H \\ CHO \end{array}$$

In general, as van't Hoff has pointed out, the total number of active forms of sugars of the same structural formula containing n asymmetric carbon atoms is 2^n.

On mild oxidation of the aldehydic group of the aldoses, optically active hydroxy-monocarboxylic acids corresponding to each active form are produced, but if by further oxidation the terminal primary alcoholic group is also converted to an acid, or if the original monosaccharide is reduced to a polyhydric alcohol, the number of possible forms of the respective dibasic acids or polyhydric alcohols is somewhat lessened. Thus, referring again to the tetroses, the dibasic acids formed by oxidation are:—

$$\begin{array}{l} COOH \\ H.C.OH \\ H.C.OH \\ COOH \\ (a) \end{array} \quad \begin{array}{l} COOH \\ H.C.OH \\ HO.C.H \\ COOH \\ (b) \end{array} \quad \begin{array}{l} COOH \\ HO.C.H \\ H.C.OH \\ COOH \\ (c) \end{array} \quad \begin{array}{l} COOH \\ HO.C.H \\ HO.C.H \\ COOH \\ (d) \end{array}$$

Of these, only (*b*) and (*c*) are optically active, and are, in fact, the *d* and *l* forms of tartaric acid, whilst (*a*) and (*b*) are identical and represent the "internally compensated" or *meso*-tartaric acid. So that there are only three stereoisomeric dibasic acids (and also tetrahydric alcohols) derived from the four tetroses, and of these only two are optically active.

Some of the possible stereoisomerides in the simpler aldomonosaccharides, with their related dibasic acids or polyhydric alcohols, are in number as follows:—

Aldoses.	Number of Asymmetric Atoms.	Number of Stereo-isomerides.	Corresponding Stereoisomeric Alcohols or Dibasic Acids.		
			Total No.	Optically Active.	Meso-compounds.
Diose .	0	1	1	0	0
Trioses .	1	2	1	0	0
Tetroses .	2	4	3	2	1
Pentoses .	3	8	4	2	2
Hexoses .	4	16	10	8	2
Heptoses .	5	32	16	12	4

Comparatively few of these sugars are found in nature, but tetroses, pentoses and all the hexoses except two are now known in active

forms corresponding to those predicted by stereochemical theory; those not occurring naturally have been produced synthetically, together with their reduction and oxidation products, by methods which will receive attention later in this monograph.

It would be impossible within the limits of a brief monograph to deal at length with the carbohydrates generally. In the following account, glucose will be taken as a typical sugar, and its properties and inter-relationships will be considered more particularly with reference to their biochemical importance. The disaccharides and glucosides will be dealt with in a similar manner. Those who desire fuller information should consult the comprehensive works compiled by Lippmann and by Maquenne.

In discussing the various problems associated with the carbohydrates, the writer will strive to indicate the alternative views which have been advanced. He will, however, endeavour to develop the subject as far as possible as a logical whole, rather than leave the reader undecided at every turn. Such a method of treatment is more likely to stimulate inquiry by giving a picture of the present attitude of workers towards the various problems which the carbohydrates present.

CHAPTER I.

GLUCOSE (DEXTRO-GLUCOSE OR DEXTROSE).

IT has been customary to speak of this sugar as *grape sugar* to distinguish it from cane sugar and on account of its occurrence in the juice of the grape and of other ripening fruits in association with fructose (laevulose). The two hexoses are probably derived from pre-existent cane sugar, as the three sugars are nearly always found together and as cane sugar is easily resolved into glucose and fructose by hydrolysis :—

$$\underset{\text{Cane Sugar.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Fructose.}}{C_6H_{12}O_6}$$

Glucose is also formed from other more complex sugars when these are broken down by hydrolysis with the assistance of the appropriate enzymes or of acids—for example, from milk sugar or lactose, malt sugar or maltose, starch and cellulose. It is easily prepared from starch by the action of diluted sulphuric acid and is therefore to be purchased at small cost. It separates from an aqueous solution with a molecule of water of crystallisation, but this is held only loosely, as the anhydrous substance may be crystallised from dilute alcohol. Unlike cane sugar, it never separates in well-defined clear crystals from either water or alcohol, but is usually met with as a crystalline powder.

Constitution.

Glucose is represented by the molecular formula $C_6H_{12}O_6$. Five of the six atoms of oxygen are to be regarded as present in the alcoholic form, as hydroxyl (OH); the sixth under certain conditions manifests aldehydic functions. Thus, when acted upon by metallic hydroxides, glucose forms compounds which resemble the "alcoholates"; and it is converted by acids, acid anhydrides and chlorides, into ethereal salts or esters such as the following :—

$C_6H_7O(NO_3)_5$	$C_6H_7O(O \cdot CO \cdot CH_3)_5$	$C_6H_7O(O \cdot CO \cdot C_6H_5)_5$
Glucose pentanitrate.	Glucose pentacetate.	Glucose pentabenzoate.

On reduction, it takes up two atoms of hydrogen and is converted into a hexahydric alcohol; on oxidation, it yields the monobasic acid, gluconic acid, $C_5H_6(OH)_5 \cdot CO \cdot OH$; when heated with a concentrated

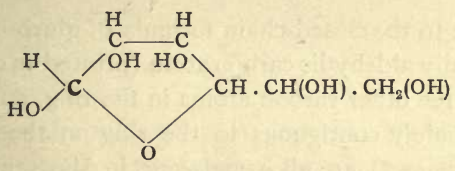
solution of hydrogen iodide, it loses the whole of its oxygen and is converted into an iodohexane, $C_6H_{13}I$, which itself is a derivative of normal hexane, $CH_3 . CH_2 . CH_2 . CH_2 . CH_2 . CH_3$.

On account of the stability of glucose, it is to be assumed that each hydroxyl group is associated with a different carbon atom; as glucose is a derivative of *normal* hexane, the constitutional formula of the aldehydic form may be written in the following manner:—

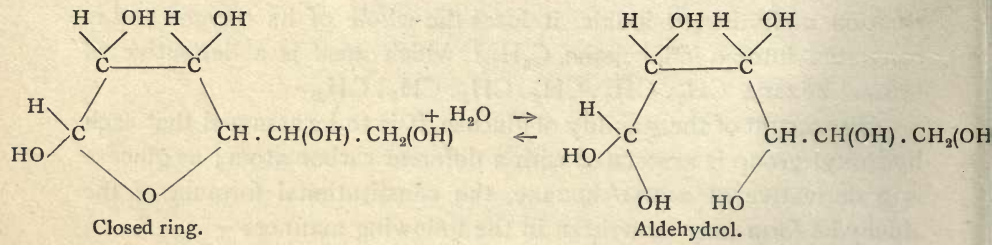
$$CH_2(OH) . CH(OH) . CH(OH) . CH(OH) . CH(OH) . CHO$$

But it was long a matter of remark that glucose, as a rule, is far less active than was to be expected, assuming it to be an hydroxyaldehyde. The difficulty was removed when Tollens, in 1883, proposed to represent it by a formula in which four of the carbon atoms are included in a ring, together with a single oxygen atom.

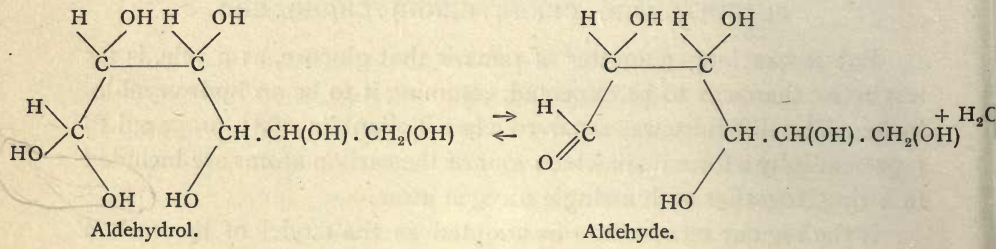
If the regular tetrahedron be adopted as the model of the carbon atom and it be supposed that the four affinities are directed towards its four solid angles from the centre of a sphere within which the tetrahedron is inscribed, the direction of the affinities is such (109° 24′) that on uniting four such tetrahedra together and interposing as representative of the oxygen atom a ball with two affinities arranged in about the same directions as the two carbon affinities, a closed system or ring is formed almost naturally, in which there is no strain, the internal angles being practically those in a regular pentagon, thus:—



This symbol has been very widely adopted, as it is in general accordance with the interactions of glucose. Fischer has stated his acceptance of it in preference to the aldehyde formula. It is the representation in a plane surface of a solid model of glucose made by combining tetrahedra in the conventional manner. The reader is advised strongly to construct such a model himself to enable him to follow the argument developed in this chapter. The behaviour of glucose as an aldehyde is accounted for if it be assumed that, when the ring is ruptured by hydrolysis, the closed-chain form passes into an aldehydrol and this in turn into the aldehydic form in the following manner:—



Closed ring. Aldehydrol.

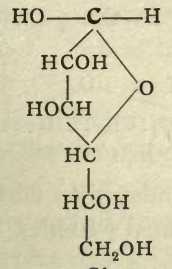


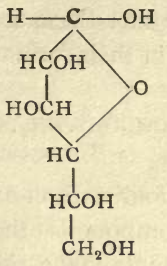
Aldehydrol. Aldehyde.

This action being reversible, it is to be supposed that when an agent such as phenylhydrazine,[1] which will act upon an aldehyde, is added to the aqueous solution, the small amount of aldehydrol present is attacked and removed; the equilibrium is thereby disturbed, but is rapidly restored by the formation of a fresh quantity of the aldehydrol, which in turn disappears but only to have its place taken by a further quantity. Ultimately the whole becomes converted into the aldehydic derivative.

On reference to the closed-chain formula of glucose, it will be seen that the potentially aldehydic carbon atom (printed in clarendon type), as well as the three other carbon atoms in the ring, and also the atom which is immediately contiguous to the ring on the right-hand side of the formula (page 7), are all *asymmetric*, in the sense that each of them is associated with four different radicles, or, in other words, a fifth asymmetric carbon atom has arisen in this formula. *Consequently the closed-chain form of glucose may be written in either of two ways*, depending on the arrangement of the groups around this atom, printed here in clarendon, thus :—

[1] See Chapter II., page 49. It is quite possible that the closed-ring form of glucose will interact directly with phenylhydrazine without the butylene oxide ring becoming opened. In such case it is unnecessary to assume the presence of any aldehydrol at all in solution.

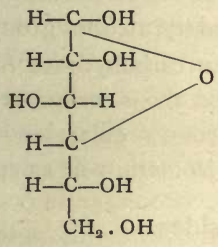

α-Glucose.


β-Glucose.

This conclusion, though in general agreement with the behaviour of glucose, does not embrace all the known facts which, as indicated on page 13, go to show that glucose may react in yet other isomeric forms owing to the presence of other cyclic systems containing only two or three carbon atoms united with the oxygen atom.

The two methyl glucosides are to be regarded as the methyl derivatives of these two stereoisomeric forms of glucose.

Nomenclature.



Carbon Atoms.		Alcohols.
1		
2	α	Secondary
3	β	Secondary
4	γ	—
5	δ	Secondary
6	ε	Primary

In order to avoid confusion it is necessary to be clear as to the nomenclature adopted. Two systems are in use: the carbon atoms may be numbered commencing with the terminal, potentially aldehydic, active carbon atom, and the formula is usually written with this upwards. Alternatively they may be designated by means of Greek prefixes in the manner customary for aliphatic acids so that the carbon atom next the potentially aldehydic atom becomes *α* and carbon number 4 is *γ*. The prefixes *α*, *β*, etc., are also commonly used by chemists to distinguish isomerides, the modifications being usually named in the order of their discovery. In the sugar group the prefixes *α* and *β* have come to acquire a special meaning as indicating the configuration of derivatives attached to carbon 1: accordingly, their use in the more general sense will be restricted as much as possible.

It is a characteristic property of *γ*-hydroxyacids to lose water very readily, forming ring compounds containing four atoms of carbon and

one of oxygen: these are termed γ-lactones as the γ-carbon atom is concerned in their formation. Thus—

$$CH_2(OH).CH_2.CH_2.CO_2H = H_2O + CH_2.CH_2.CH_2.CO$$

γ-Hydroxybutyric acid. γ-Butyrolactone.

Similarly, four carbon-oxygen ring compounds, when derived from γ-hydroxy compounds other than acids, are named γ-oxides. The ring is termed a pentaphane ring.

It is, however, preferable again to avoid the use of the Greek prefix and to denote the character of the ring structure by the terms, ethylene, propylene, or butylene oxide instead of as α-, β- or γ-oxides, as was done in the former edition.

An alternative, advocated by Hudson, is the use of the word "cyclo" with a Greek prefix to show the nature of the ring, e.g. methyl-α-cyclo-glucose or methyl-γ-cyclo-glucose. Acree has suggested the term lactonyl to indicate an aldehyde group that has formed a lactone-like ring, and Hudson uses the symbol < to denote this. This symbol is useful in expressing the structure of the disaccharides.

The carbon atom and the attached H and OH radicles are often referred to collectively as primary or secondary alcohol groups.

The prefix *epi* is used to denote the new carbohydrate formed by the interchange of the H and OH groups on the α-carbon atom; thus mannose becomes epiglucose, ribose becomes epiarabinose. The change is spoken of as epimerism, and the isomeric pair as epimerides.

The Methyl Glucosides.

In considering the structure of glucose, the compounds which deserve attention in the first place are the two isomeric methyl glucosides (α and β), which are formed by the interaction of glucose and methylic alcohol under the influence of hydrogen chloride. These compounds are the prototypes of the natural glucosides. They were discovered by Emil Fischer in 1893. He prepared them by dissolving glucose in cold methylic alcohol saturated with dry hydrogen chloride gas. After several hours, when it had lost all cupric reducing power, the mixture was neutralised with lead carbonate. Crystals of the α-compound were obtained on concentrating the solution; the β-compound was isolated later from the mother liquor, and was first obtained crystalline by Van Ekenstein.

The methyl glucosides differ considerably from glucose, more particularly in never behaving as aldehydes; and their rotatory power in solution is the same in a freshly-prepared solution as it is in one which

has been kept for some time, which is not the case with glucose. They are undoubtedly formed by the introduction of methyl, in place of an atom of hydrogen, in the hydroxyl group attached to the carbon atom which exercises aldehydic functions in the open-chain form of glucose. It is to be noted that the introduction of methyl in this position has the effect of rendering the ring far more stable than it is in glucose, as it is to be supposed that compounds such as phenylhydrazine, and oxidising agents such as Fehling's solution, are without action because the glucosides do not undergo hydrolysis in solution in the way that glucose does.

The two glucosides are distinguished by the arbitrary prefixes *a* and *β*, their physical properties being as follows:—

	Melting-point.	Rotatory Power.
a-Methyl glucoside	165°	+ 157°
β-Methyl glucoside	104°	− 33°

They are both colourless crystalline substances, the *a*-isomeride crystallising usually in long needles, the *β*-isomeride in rectangular prisms.

When hydrolysed by acids they yield methyl alcohol and glucose. At ordinary temperatures hydrolysis, even by moderately strong mineral acids, proceeds but slowly; and if it be desired to study the course of hydrolysis it is advisable to work at elevated temperatures, say 70° to 80° C. As in other chemical reactions, the hydrolytic power of acids towards glucosides increases as the temperature is raised. A convenient method of experimenting consists in mixing acid and glucoside in a closed flask immersed in a thermostat so as to maintain the required temperature. Samples of the liquid are withdrawn at stated intervals of time, rapidly cooled by immersion in ice water to check hydrolysis, and the amount of glucose formed estimated either gravimetrically or with the polarimeter. To prevent evaporation it is advisable to add a little paraffin wax to the mixture of glucoside and acid. Measurements made in this way show that a definite fraction of the glucoside present is hydrolysed in each unit of time, the course of change following what is known as the logarithmic curve. The *β* compound is attacked more rapidly than the *a*. This point will be referred to again in Chapter VI.

The methyl glucosides are also hydrolysed by enzymes, but both isomerides are not hydrolysed by the same enzyme. In fact, the action of enzymes towards the glucosides is specific, and each form requires its own particular enzyme: *a*-methyl glucoside is hydrolysed by maltase: *β*-methyl glucoside by emulsin. The enzymes act at

ordinary temperatures, preferably not above 37° C., and are far more active as hydrolytic agents than acids.

Returning to the preparation of the glucosides just described it will be noted that both forms are produced simultaneously, the *α*-isomeride predominating. When solid anhydrous glucose (*α*-glucose) is dissolved in dry methyl alcohol containing dry hydrogen chloride, the first change is its rapid conversion into a mixture of *α*- and *β*-glucose in nearly equal parts. Each of these then undergoes etherification, the primary result being a mixture of *α*- and *β*-methyl glucosides, in which the latter is slightly in excess. On standing, slow conversion of the *β*-methyl glucoside into the more stable *α*-isomeride takes place. The equilibrated mixture of the glucosides contains 77 per cent. of the *α*- and 23 per cent. of the *β*-isomeride. If, however, the solution be neutralised as soon as etherification is complete and before the isomeric changes take place, and the solvent be removed, a mixture of the two glucosides in approximately equal quantities is obtained. These may be separated by fractional crystallisation.

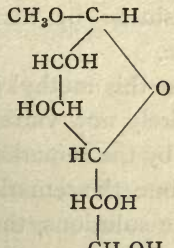
Such a process is somewhat tedious when *β*-methyl glucoside is the object of the preparation, and it is more convenient to make use of biological methods. On treatment with yeast, which contains the enzyme maltase, the *α*-methyl glucoside is hydrolysed to glucose and methyl alcohol, and the glucose is removed by fermentation, so that *β*-methyl glucose, which is not attacked by yeast, alone remains, and can be isolated and purified.

When, on the other hand, *α*-methyl glucoside is desired, the action of the acid is allowed to continue until equilibrium is attained, and, after crystallisation of some quantity of the *α*-methyl glucoside, the mother liquors are again heated with a little acid. This has the effect of causing the *β*-glucoside present to be converted into *α*-glucoside until equilibrium is again reached, when 77 per cent. of the total solid present is *α*-glucoside, and in consequence a further quantity of *α*-glucoside crystallises on removal of the solvent.

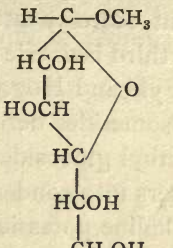
Fischer employs an alternative method, which consists in heating the alcoholic glucose solution with very little acid in an autoclave. It is then not necessary to neutralise before crystallisation of the *α*-glucoside.

Maquenne has prepared *β*-methyl glucoside by the action of methyl sulphate and sodium hydroxide on glucose dissolved in water. It is stated that the *β*-isomeride alone is formed under these conditions, but the quantity obtained is not large.

The two methyl glucosides are regarded as stereoisomeric γ or butylene oxides, and have the following structural formulæ:—



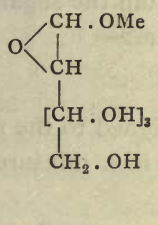
α-Methyl glucoside.



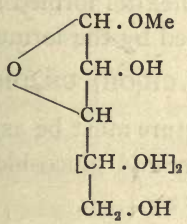
β-Methyl glucoside.

In practically every respect the above formulæ may be regarded as satisfactory and consistent with the properties of the isomeric glucosides, their different solubilities, rotations, rates of hydrolysis, and their behaviour towards enzymes. Considering the cyclic structures possible in glucose it is obvious that the ring forming oxygen can occupy other positions than the γ or butylene oxide position so far assumed.

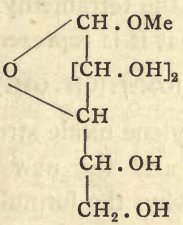
The alternatives are :—



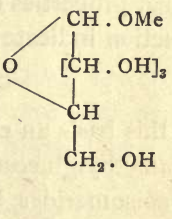
Ethylene oxide.



Propylene oxide.

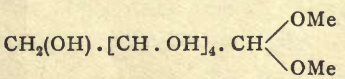


Butylene oxide.



Amylene oxide.

The remarkable ease with which fructosides are hydrolysed suggests that these compounds are constituted on a plan different from that of the glucosides, and careful experimental work has indeed shown that further isomerides, both of the methyl glucosides and other derivatives of glucose and similar sugars, exist although these are somewhat indefinitely characterised. Fischer originally showed that the reaction between glucose and methyl alcohol containing 1 per cent. of hydrogen chloride yields, in addition to the two crystalline methyl glucosides, a considerable amount of a syrup which has hitherto not been purified and has been regarded as glucose dimethylacetal, $C_8H_{18}O_7$, i.e.



or as an uncrystallisable mixture of the α- and β-methyl glucosides. He found in 1914 that the syrup distils without decomposition in a high vacuum, and has the composition, $C_7H_{14}O_6$, of a methyl glucoside. It is stable to alkalis, Fehling's solution and hot water, is hydrolysed

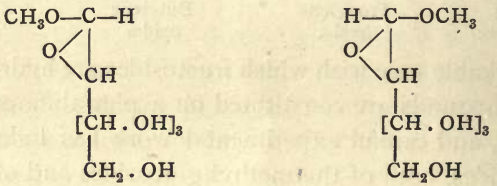
by acids but scarcely attacked by emulsin or by maltase. It is obviously a third isomeric methyl glucoside.

Irvine, Fyfe and Hogg have shown that this methyl glucoside is a mixture of isomerides derived from an entirely new variety of glucose. The new methyl glucoside is characterised by the remarkable ease with which it enters into condensation with acetone, the remarkable capacity to reduce alkaline potassium permanganate solutions, the tendency to unite with one atomic proportion of oxygen to give a neutral product, and the ready auto-condensation of this oxy-compound to give a product allied to the disaccharides.

When methylated by the silver oxide method a new tetramethylmethylglucoside is formed which behaves as a mixture of isomerides. When hydrolysed a new liquid, tetramethylglucose, is obtained which is lævo-rotatory and behaves as a derivative of a much more reactive form of glucose than the α or β variety. It fails to form a phenylosazone, being resinified by phenylhydrazine and acetic acid. The optical properties of the tetramethyl hexitol formed from the sugar by reduction indicate that it is represented by the formulæ:—

$$CH_2(OMe).[CH.OMe]_3.CH(OH).CH_2(OH)$$

On this basis an ethylene oxide structure must be assigned to the new tetramethylglucose, and the new methyl glucoside is a mixture of stereoisomerides, having the formulæ:—



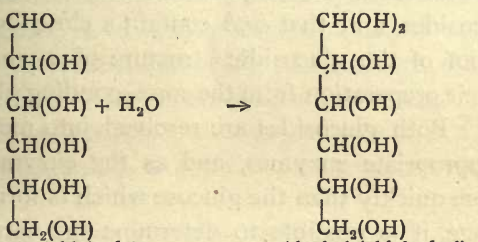
The new glucose itself has not yet been isolated in the free state but it is evident that its reactivity far exceeds that of α- or β-glucose.

Nef, as the result of his investigations on the β and γ lactones of the sugar acids, considers that the isomerism of the methyl glucosides does not depend on the position of the methyl group. He formulates the α-glucoside with a butylene oxide ring and the β-glucoside with a propylene oxide ring.

Mutarotation—The Isomeric Forms of Glucose.

The hypothesis that there are two stereoisomeric forms of glucose is the only one hitherto proposed which affords a satisfactory explanation of a peculiar property, characteristic of glucose and other sugars manifesting aldehydic functions, now known as *mutarotation* or *multirotation* (but formally termed *birotation*); namely, the optical rotatory power of the freshly dissolved substance changes gradually, sometimes increasing, but more usually falling, until a constant value is reached. The term *birotation* was introduced because the rotatory power of glucose in solution is about twice as great when it is freshly dissolved as that which it eventually assumes. The change takes place very slowly when highly purified materials are used, but almost immediately if a small quantity of alkali be added. The phenomenon was first observed by Dubrunfaut in 1846 and ascribed by him to purely physical causes. The subject has of recent years caused a good deal of discussion, and it is simplest to deal with the views that have been advanced in historical sequence.

E. Fischer, in 1890, noticed that the optical rotatory power of certain lactones closely related to the sugars underwent change in solution as the lactone became hydrolysed to the corresponding acid. He therefore ascribed the change which occurs with glucose to a like addition of a water molecule, and assumed that the glucose (aldehyde) underwent conversion into a heptahydric alcohol (aldehydrol) of lower rotatory power:—



Glucose (aldehyde). Alcohol (aldehydrol).

The subject assumed a new aspect when it was shown by Tanret, in 1896, that besides the anhydrous and hydrated forms of glucose other isomeric anhydrous modifications could be obtained. He described an α-glucose ($[\alpha]_D + 110°$), the initial rotatory power of which fell gradually to $[\alpha]_D + 52{\cdot}5°$; further, a β-glucose[1] of low initial

[1] Tanret actually termed the substance represented above as β-glucose γ-glucose and designated γ-glucose as β-glucose. The terms have been altered to bring them into agreement with the nomenclature adopted.

rotatory power ($[\alpha]_D + 19°$), increasing to $[\alpha]_D + 52{\cdot}5°$ in solution ; and, lastly, a γ-glucose ($[\alpha]_D + 52{\cdot}5°$) of unalterable rotatory power in solution. The three supposed isomerides were isolated by allowing glucose solutions to crystallise under different conditions—α-glucose separated at ordinary temperatures from solutions in 70 per cent. alcohol, and β-glucose from aqueous solutions at temperatures above 98° C.; γ-glucose was obtained by precipitating a concentrated aqueous solution of glucose with alcohol. α-Glucose hydrate crystallises from aqueous solutions at the ordinary temperature. When powdered anhydrous glucose is added to water, it immediately undergoes hydration before passing into solution.

The behaviour of these isomeric forms does not fit in with the theory that the mutarotation is due to the conversion of an aldehyde into an aldehydrol; moreover, the increase in rotatory power from β- to γ-glucose has also to be explained.

Tanret, Lippmann and others suggested that some forms of glucose have a closed-ring structure, as proposed by Tollens, and that in solution these are completely converted into the isomeric aldehyde.

A more fruitful suggestion was made by Simon who drew attention to the optical behaviour of α- and β-glucose in relation to that of the isomeric methyl glucosides of which the structure was known :—

	$[\alpha]_D$		$[\alpha]_D$
α-Methyl glucoside	+ 157°	α-Glucose	+ 105°[1]
β-Methyl glucoside	− 33°	β-Glucose	+ 22°

He suggested that the α- and β-glucoses are homologues of the α- and β-methyl glucosides, and that *both* contain a closed oxygenated ring.

Direct proof of the glucosidic structure of α- and β-glucose was afforded by their preparation from the corresponding glucosides effected by the writer. Both glucosides are resolved into methyl alcohol and glucose by appropriate enzymes, and as the enzymes condition the hydrolysis more quickly than the glucose which is formed can undergo isomeric change, it is possible to determine the nature of the sugar which is formed initially. In practice, this is done by preparing a clear solution of glucoside and enzyme, allowing hydrolysis to proceed for a short time and then observing the optical rotatory power of the solution before and after the addition of a drop of ammonia, which hastens the rate of the isomeric change, and therefore has the effect of establishing equilibrium almost immediately. As a glucose of high initial rotatory power was obtained from α-methyl glucoside, and one

[1] The numerical values are Simon's.

of low initial rotatory power from the β-glucoside, it is clear that α- and β-glucose correspond respectively to the α- and β-glucoside.

It remains to establish the nature of Tanret's γ-glucose, which he, as well as Simon and Lippmann, regarded as a third isomeride, ascribing the mutarotation of α- and β-glucose to their complete conversion into the isomeric aldehyde.

The change in rotatory power of glucose was shown to be a process of reversible isomeric change by Lowry in 1899. Lowry subsequently (1903) concluded that not only are α- and β-glucose isodynamic compounds, but that Tanret's γ-glucose is a mixture in which these two compounds are present in equilibrium.

On concentration of the solution of such an equilibrated mixture, a point is reached when one of the constituents crystallises out from the saturated liquid. The mixture in solution is consequently thrown out of equilibrium; but as this happens a change takes place spontaneously to restore the equilibrium—β passing into α, or *vice versâ*. A solution of glucose containing α and β forms can therefore be made to yield wholly α- or wholly β-glucose on concentration, according to the temperature at which crystallisation takes place. The α form, which is then the less soluble, is that obtained at lower temperatures; but above 98°, the β form, being the less soluble at the higher temperature, alone separates. Were the change into aldehyde complete, as Simon and Lippmann suggest, it would be impossible by mere crystallisation to convert this into α-glucose.

Tanret (1905) has accepted the conclusion that there are but two isomerides of glucose, corresponding to the α- and β-methyl glucosides, and that his supposed third modification is an equilibrated mixture of these two forms. He has calculated from the rotatory power $[\alpha]_D + 110°$ of the pure α and $[\alpha]_D + 19°$ of the pure β form that the proportion in which these are in equilibrium is $\alpha = 37$ per cent., $\beta = 63$ per cent. in a 10 per cent. solution, and $\alpha = 40$, $\beta = 60$ per cent. in a concentrated aqueous solution.

By means of solubility determinations Lowry finds 52 per cent. of the α form to be present in saturated solutions of glucose in methyl alcohol: the proportion of α decreases as the amount of water increases, amounting to 40 per cent. in the mixture $MeOH + H_2O$. He does not, however, interpret the remaining 60 per cent. of sugar present in solution as β-glucose, but considers that some quantity of the aldehyde form is also present.

Conclusive proof that the mutarotation is caused by a balanced reaction between the α and β forms of the sugar is afforded by the

numerical equality of the velocity coefficients of their mutarotation which have been determined over a range of temperature from 0° to 40°.

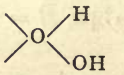
Behrend finds that *α*-glucose can exist in contact with boiling ethyl or isobutyl alcoholic solutions, or as the monohydrate in contact with aqueous solutions. From the solution in boiling pyridine a monopyridine salt of *β*-glucose separates, which on exposure rapidly loses pyridine. This forms a convenient method of preparing *β*-glucose, which, according to Behrend, has m.p. 148°-150°, $[\alpha]_D + 20{\cdot}7°$.

Glucose as purified by crystallisation from dilute methyl alcohol is almost invariably a mixture of the different forms. To obtain a homogeneous substance the solid is soaked during several days or weeks with the solvent, at a constant temperature, until the whole of the *β*-sugar present has been converted into the *α*-isomeride (Lowry).

According to Hudson and Dale, acetic acid of various concentrations is the most suitable solvent for the recrystallisation of *α*- and *β*-glucose. To obtain *α*-glucose 2 parts of the sugar are dissolved in 1 part of water and mixed with 4 parts of glacial acetic acid; crystallisation is allowed to take place at the ordinary temperature. The best method of preparing *β*-glucose is as follows: 10 parts of glucose are dissolved in 1 part of water on a water bath and 12 parts of glacial acetic acid heated to 100° are added. The whole is well mixed and removed from the water bath, when crystallisation at once commences. After four such crystallisations pure *β*-glucose is obtained.

Hudson gives $[\alpha]_D + 110°$ for *α*-glucose and + 19° for *β*-glucose.

When the mixture of alcohol and water is sufficiently dilute glucose crystallises as hydrate, the transformation from anhydrous glucose to hydrate being clearly visible to the eye as the sugar changes from a fine powder to a hard cake of glistening crystals. Glucose hydrate undoubtedly has the structure of the oxonium hydroxide:—



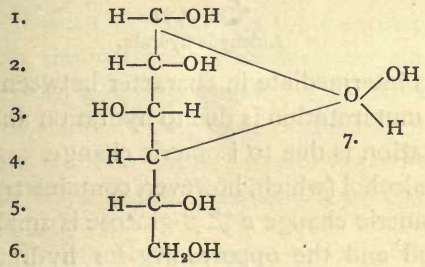
It is characteristic of the carbohydrates that their optical rotatory power is altered, in some cases very considerably, by changes of concentration of the sugar. On the hypothesis that actually there is present in solution a mixture of two isomerides in equilibrium, it is obvious that the change in question will disturb the equilibrium in one or the other direction. In the case of glucose temperature has hardly any influence, but the rotation is greater in more concentrated solutions. When these are diluted the rotatory power returns to the lower value only slowly, corresponding with the gradual establishment of the new

equilibrium. The rotation of fructose is very greatly influenced by change of temperature. The effect of salts in altering the rotatory power is also in part due to their concentration effect tending to alter the position of the equilibrium.

The knowledge of the mutarotation of glucose and fructose, particularly when liberated from sucrose, has been materially advanced by Hudson in a series of papers commenced in 1908, some years subsequent to the definite proof of the nature of mutarotation by Armstrong and Lowry.

Hudson draws attention to the recognition by O'Sullivan and Tompson in 1890 that the earlier polarimetric measurements of the inversion of sucrose by invertase were vitiated by a systematic error due to the fact that the glucose formed is initially in a mutarotatory condition. The optical rotation only gives a true measure of the amount of inversion after the addition of a drop of alkali.

The conductivity of *a*-glucose in presence of boric acid decreases during mutarotation, whilst in the case of the *β* form the reverse is the case. Böeseken, in the light of his experience with polyhydroxy alcohols, interprets this fact as a proof that in *a*-glucose the hydroxyl radicles attached to carbons 1 and 2 are on the same side of the molecule and assigns a configuration to *a*-glucose which is the reverse of that pictured on page 9. In drawing this conclusion he has overlooked the conditions developed when water is added to the oxygen atom of the ring—



From the formula above it will be seen that the pair of hydroxyls 1 , 7 may be responsible for the change in conductivity just as much as the pair 1 , 2.

The question has been settled by Irvine and Steele from the study of the mutarotation and conductivity of tetramethylglucose. Proof is afforded that when dissolved in water this exists in the first place as a monohydric alcohol. The marked increase in conductivity in the presence of boric acid, observed as mutarotation proceeds, shows that an additional hydroxyl group becomes attached to the sugar, and this

can only take place at the oxygen atom of the ring (position 7). The subsequent elimination of water may take place in either of two ways to generate the *a* or *β*-sugars. The final equilibrium is :—

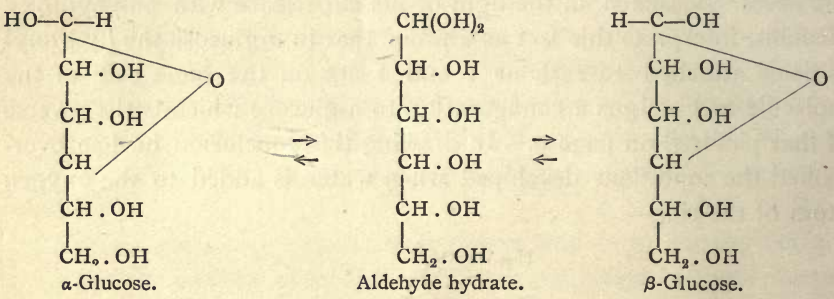
a form ⇄ oxonium hydrate ⇄ *β* form.

It follows that Böeseken's deductions cannot be accepted as proof of the structure of *a* and *β*-glucose.

Isomeric Change.—*a* ⇄ *β*-Glucose.

It remains to discuss the mechanism of the isomeric change *a* ⇄ *β*-glucose. Two rival explanations have been advanced which differ really only in one respect: Lowry considers the formation of the aldehyde or its hydrate, which involves the opening of the ring, to be an intermediate stage in the process; E. F. Armstrong, however, has formulated the change as taking place without any disruption of the γ-oxide ring.

According to Lowry's view, the change is represented by the scheme of equilibrium :—

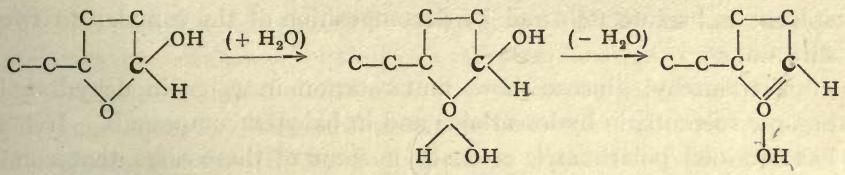


a-Glucose. Aldehyde hydrate. *β*-Glucose.

This scheme is intermediate in character between Fischer's former view (p. 15), that mutarotation is due to hydration and the more recent view that mutarotation is due to isomeric change.

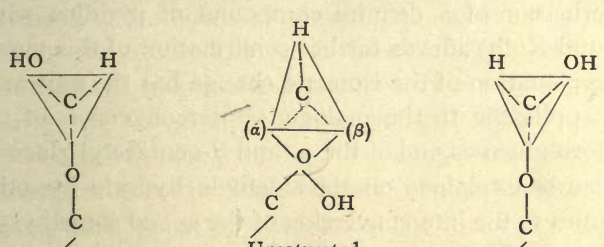
In anhydrous alcohol (which, however, contains traces of water) the velocity of the isomeric change *a* ⇄ *β*-glucose is small, but it increases as water is added and the opportunity for hydration is increased. Lowry takes the view that an aqueous solution of glucose contains a considerable proportion of aldehyde (open-chain form), in addition to *a*- and *β*-glucose (closed-ring forms), whereas in alcoholic solution there is little or no aldehyde.

E. F. Armstrong considers the first stage in the process to be the formation, by the addition of water, of the oxonium hydrate, from which, by the elimination of water in another manner, an unsaturated compound results. This is illustrated in the following scheme, in which only the carbon skeleton of the pentaphane ring is indicated :—



Glucose. Oxonium hydrate. Unsaturated compound.

It is possible to add the elements of water to this unsaturated bond in either of two ways, giving rise to the α- and β-glucoses respectively, or their oxonium hydrates. If this stereoisomerism is pictured in plane projection in the conventional manner with reference to the terminal carbon atom[1] (in Clarendon type in the preceding diagram), the simultaneous formation of both isomerides according as the hydroxyl group re-enters into combination with the terminal carbon atom at the respective valency points marked (α) and (β) in the unsaturated oxonium compound is evident:—



1st position = α-Glucose. Unsaturated oxonium compound. 2nd position = β-Glucose.

Lowry's view that an aldehydrol is the intermediate compound is not consistent with the increase in conductivity during mutarotation and may therefore be dismissed, and the evidence in favour of the oxonium theory may now be regarded as conclusive.

The mechanism of mutarotation probably varies with the particular solvent employed, but it depends essentially on combination between

[1] The asymmetric carbon atom in Clarendon type has attached to it the four radicles—(1) hydrogen, (2) hydroxyl, (3) the pentaphane oxygen, (4) a carbon atom of the ring. The stereoisomerism of α- and β-glucose is explained above as due to the interchange in the relative positions of the hydrogen and the pentaphane oxygen. This relationship is awkward to picture in plane formulæ; it is therefore more convenient to represent the stereoisomerism as due to the interchange in the relative positions of the hydrogen and hydroxyl radicles, as is done for example in the formula on previous pages. Reference to a solid model will show that this comes to exactly the same in the end, as the carbon atom in engaging with the pentaphane oxygen in its α or β position is necessarily rotated, so that a projection of the solid tetrahedron viewed in plan will show hydrogen alternately on the right and left of hydroxyl. It is almost essential to consult a model if a full understanding of the difference between α- and β-glucose and also between glucose and galactose is desired.

solvent and solute followed by decomposition of the complex in two directions.

Tetramethyl glucose shows mutarotation in water, in dehydrated organic solvents, in hydrocarbons and in halogen compounds. Irvine has obtained polarimetric evidence in some of these cases that complexes of the oxonium type are formed. Whereas in aqueous solutions the change is promoted by alkaline catalysts, in acetone an acid catalyst appears necessary before the change takes place, probably because change is only promoted when the solvent is partly enolised and may thus combine with the sugar. Examples of this are afforded by dimethyl-β-glucose (Irvine and Scott) and by the anilides of alkylated sugars (Irvine and McNicoll).

No doubt the same explanation—combination with the enolised solvent—will be found to apply to the mutarotation in anhydrous formamide solution studied by Mackenzie and Gosh.

The formation of a definite compound of pyridine with glucose (Behrend and Roth) affords further confirmation of this view.

This explanation of the isomeric change has the advantage that it is equally applicable to the analogous interconversion of the α- and β-acetochloro glucoses and of the α- and β-pentacetyl glucoses, neither of which can be explained on the aldehyde hydrate hypothesis; and it also applies to the interconversion of the α- and β-methyl glucosides. In this last case Fischer has assumed that an intermediate compound of the acetal type is produced and the pentaphane ring is opened—a scheme identical with that just described as subsequently advocated by Lowry. The first product of the action of dry methyl alcohol containing 1 per cent. of hydrogen chloride on glucose at the ordinary temperature is a syrup differing from either glucoside. This could not be analysed, but was regarded by Fischer as glucose dimethyl-acetal. It has now been shown to be probably the ethylene oxide form of methyl glucoside.

Measurements of the velocity of their transformation made by Jungius led him to the conclusion that the two glucosides are directly convertible into each other and that it is very improbable that an acetal is formed. Further, the reversible conversion of the α- and β-tetramethyl methyl glucosides takes place at temperatures of 110°-150° independently of the nature of the solvent used: a result which excludes the intermediate formation of a compound of an acetal type.

The isomeric change of one series of glucose derivatives into the other has been formulated in the foregoing on the hypothesis that

additive oxonium compounds are formed in which the lactonic oxygen displays quadrivalency. Indeed no other explanation is applicable to all the transformations observed in the glucose series. Such additive oxonium compounds are well known to be formed in other cases, such as dimethylpyrone (Collie and Tickle). Irvine and Moodie have brought forward evidence to show that tetramethyl glucose forms an oxonium derivative with isopropyl iodide. The presence of the etheric groups in the alkylated sugar apparently increases the basicity of the butylene-oxidic oxygen atom, and so makes the identification of the oxonium compound possible.

From the biological point of view, the fact that glucose exists in solution not as a single substance but as an equilibrated mixture of stereoisomeric butylene oxide forms, readily convertible into one another, is of fundamental and far-reaching importance. If one of the stereoisomerides is preferentially metabolised in the plant or animal, in the course of either synthetic or analytic processes, the possibility of controlling the equilibrium in the one or other direction, so as to increase or limit the supply of this form, places a very delicate directive mechanism at the disposal of the organism. This question is undoubtedly one which demands the close attention of physiologists.

The speeds of mutarotation of most of the sugars are indicated in the following table, given by Hudson :—

TABLE I.—The Velocity-Coefficients of the Mutarotation of the Sugars in Water at 20°.

Sugar.	$k_1 + k_2 = \frac{1}{t} \log \frac{r_0 - r_\infty}{r - r_\infty}$ (Minutes and Decimal Logarithms).
Fructose	0·082
Lyxose	0·065
Rhamnose	0·039
Arabinose	0·031
Fucose	0·022
Xylose	0·021
Mannose	0·0190
α-Glucoheptose	0·0122
Galactose	0·0102
Melibiose	0·0088
Maltose	0·0072
Glucose	0·0065
Cellose	0·0047
Lactose	0·0046

The initial and final solubilities of most of the crystalline sugars are summarised in the following table likewise due to Hudson :—

TABLE II.—SOLUBILITIES OF SUGARS AT 20°.

Sugar.	Formula.	Solvent.	Grams of Anhydrous Sugar in 100 c.c. Solution.	
			Init. Sol.	Final Sol.
α-Arabinose	$C_5H_{10}O_5$	80 per cent. alcohol	0·74	1·94
β-Cellose	$C_{12}H_{22}O_{11}$	20 ,, ,,	3·2	4·7
β-Fructose	$C_6H_{12}O_6$	80 ,, ,,	13·4	27·4
,,	$C_6H_{12}O_6$	95 ,, ,,	1·8	4·2
,,	$C_6H_{12}O_6$	Methyl alcohol	5·2	11·1
α-Galactose	$C_6H_{12}O_6$	60 per cent. alcohol	1·1	3·1
,,	$C_6H_{12}O_6$	80 ,, ,,	0·27	0·65
β,α-Glucoheptose	$C_7H_{14}O_7$	20 ,, ,,	4·0	4·5
α-Glucose	$C_6H_{12}O_6$	80 ,, ,,	2·0	4·5
,,	$C_6H_{12}O_6$	Methyl alcohol	0·85	1·6
α- ,, hydrate	$C_6H_{12}O_6 . H_2O$	80 per cent. alcohol	1·3	3·0
β- ,,	$C_6H_{12}O_6$	80 ,, ,,	4·9	9·1
α-Lactose hydrate	$C_{12}H_{22}O_{11} . H_2O$	40 ,, ,,	1·1	2·4
α-Lyxose	$C_5H_{10}O_5$	90 ,, ,,	5·4	7·9
β-Maltose hydrate	$C_{12}H_{22}O_{11} . H_2O$	60 ,, ,,	3·0	4·75
β-Mannose	$C_6H_{12}O_6$	80 ,, ,,	2·4	13·0
,,	$C_6H_{12}O_6$	Methyl alcohol	0·78	4·4
β-Melibiose dihydrate	$C_{12}H_{22}O_{11} . 2H_2O$	80 per cent. alcohol	0·76	1·3
α-Rhamnose hydrate	$C_6H_{12}O_5 . H_2O$	Absolute alcohol	8·6	9·5
,, ,,	$C_6H_{12}O_5 . H_2O$	70 per cent. alcohol	8·2	9·6
α-Xylose	$C_5H_{10}O_5$	80 ,, ,,	2·7	6·2
Sucrose	$C_{12}H_{22}O_{11}$	80 ,, ,,	3·7	3·7
Trehalose dihydrate	$C_{12}H_{22}O_{11} . 2H_2O$	70 ,, ,,	1·8	1·8
Raffinose pentahydrate	$C_{18}H_{32}O_{16} . 5H_2O$	50 ,, ,,	1·4	1·4

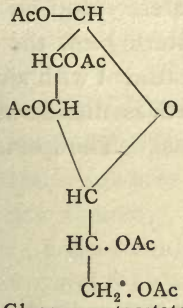
The More Important Derivatives of Glucose.

The experimental work of the last ten years has shown that most of the derivatives of glucose likewise exist in two or more forms differing in physical properties, more particularly crystalline form, optical rotatory power and melting-point. The chemical behaviour of all these substances is such that it must be assumed that the aldehydic function has disappeared, giving rise to the closed-ring structure already formulated.

Glucose Pentacetates.

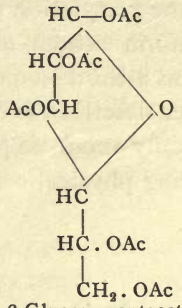
Under proper experimental conditions, all five hydroxyl groups in glucose become acetylated, the *α*- or *β*-pentacetate predominating in the product according to the method adopted. As these compounds form the starting-point for a number of syntheses, it is important to understand fully the methods of preparing them.

They have the following formulæ :—



α-Glucose pentacetate.

[Ac = C_2H_3O]



β-Glucose pentacetate.

To obtain the α-pentacetate it is necessary to acetylate glucose instantly before isomeric change can take place, since the presence of acid greatly accelerates the isomeric change from α- to β-glucose. This is done by adding anhydrous α-glucose to boiling acetic anhydride containing a small quantity of zinc chloride as catalyst. A violent action ensues, and the sugar passes into solution. The product is poured into water, which is changed from time to time to remove the acetic acid; finally the α-glucose pentacetate solidifies. The crude product contains both isomerides: it is purified by crystallisation from alcohol. The α-pentacetate predominates also when glucose is acetylated in pyridine solution at 0°.

To obtain the β-pentacetate, glucose is mixed with acetic anhydride and sodium acetate, and heated for some time at the temperature of the water bath. As the change from α- to β-glucose in this case precedes acetylation, β-glucose pentacetate predominates in the final product, and may be separated by fractional crystallisation.

The pentacetates are colourless crystalline compounds, insoluble in water and readily hydrolysed by alkaline hydroxides. When heated with acetic anhydride either form is partially converted into the other until equilibrium is attained when 90 per cent. of the α and 10 per cent. of the β form are present. Jungius has shown that this change may also be effected by adding a small amount of sulphur trioxide to a solution of the acetate in chloroform.

In the case of galactose no less than four pentacetates have been isolated.

The β-butylene-oxide form was first prepared by Erwig and Koenigs by acetylating galactose with acetic anhydride and sodium acetate. Hudson and Parker find that when this form is boiled with acetic anhydride and a little zinc chloride the α-butylene oxide isomeride is obtained to the extent of about 70 per cent. of the original

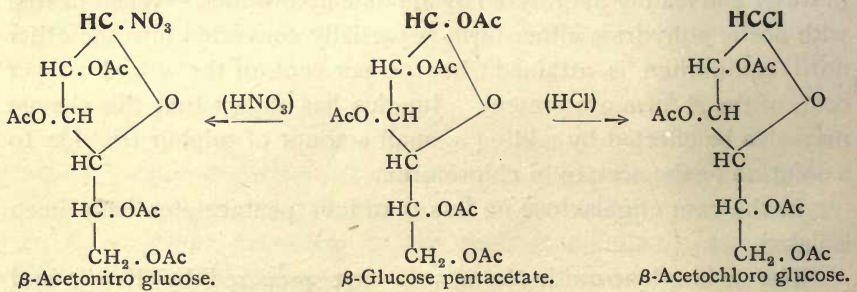
material. The usual method of separating the β-pentacetate is to pour the acetylation product into cold water. Hudson finds that the chloroform extract of this water after filtering off the β-pentacetate contains a third isomeride, and this on treatment with zinc oxide in the manner described is converted into a fourth isomeride in small quantities—only about 10 per cent. of the original. The isomerides have the following physical constants :—

TABLE III.

No.	Structure.	Melting-point.	$[\alpha]_D$.
1	β-Butylene oxide . .	142	+ 7·5°
2	α- ,, ,, . .	95·5	+ 106·0°
3	β-Ethylene oxide . .	98	− 42°
4	α- ,, ,, . .	87	+ 61°

Hudson regards his new isomerides as possessing an oxide structure other than a butylene oxide, and in the light of the foregoing pages the ethylene oxide structure may provisionally be assigned to them.

Acetochloro, Acetonitro Glucoses.—In either isomeride, one of the acetyl groups—that attached to the terminal carbon atom (in Clarendon type) linked to the pentaphane oxygen atom—is far more active than the rest. When subjected to the action of anhydrous liquid hydrogen bromide or hydrogen chloride in sealed tubes at the ordinary temperature, this acetyl group alone is displaced by halogen. In this way α-pentacetyl glucose gives α-acetochloro glucose, β-pentacetyl glucose the corresponding β-acetochloro glucose—both beautifully crystalline colourless substances. Nitric acid acts in a similar manner, causing the formation of crystalline α- and β-acetonitro glucoses :—



β-Acetonitro glucose. β-Glucose pentacetate. β-Acetochloro glucose.

Physical measurements also indicate that one of the acetyl groups is more easily detached than the others. This is proved by the fact that the rate at which the acetyl groups are removed by hydrolysis with alkali from the glucose pentacetates decreases as change pro-

ceeds; yet the tetra-acetyl methyl glucosides, which contain four similarly placed acetyl groups but lack the one contiguous to the pentaphane oxygen, are hydrolysed by alkali at a rate which is constant throughout the whole change.

Hudson in a similar manner has obtained a new acetochloro galactose from his third β-ethylene oxide pentacetate, and it is obviously possible to obtain a whole series of ethylene oxide derivatives of galactose in this manner.

The chloro-, bromo- and nitro- groups are even more reactive than the acetyl group, and are easily displaced—for example, by methoxyl—on shaking a solution of the compound in anhydrous methyl alcohol with silver carbonate. The isomeric tetra-acetyl methyl glucosides thus obtained are converted, when hydrolysed by an alkali, into the corresponding isomeric methyl glucosides. These syntheses make it possible to pass from β-glucose to β-methyl glucoside through a series of β compounds and to correlate all these compounds with β-glucose.

Acetochloro and acetobromo glucose have been rendered easily accessible by a more convenient method of preparation: powdered crystalline anhydrous glucose, dissolved in about five times its weight of acetic anhydride, is boiled with half its weight of anhydrous sodium acetate for two or three hours. The product is poured into a large volume of ice-water and the crude β-glucose pentacetate is freed as much as possible from acetic anhydride by pulverisation under water and then crystallised from 96 per cent. alcohol, when it is obtained in 74 per cent. yield.

One part of the pentacetate is left with two parts of the commercial solution of hydrobromic acid in acetic acid for two hours at the ordinary temperature. Four parts of chloroform are added and the mixture shaken with twice its weight of ice-water; the chloroform extract is run off and the residue again shaken with a little chloroform, after which the united chloroform solutions are washed with water, dried over calcium chloride, and the chloroform removed under a vacuum. The oily residue is triturated with light petroleum until crystallisation sets in and subsequently the collected crystals are rapidly recrystallised from a little amyl alcohol, washed with light petroleum, and stored in a vacuum over soda-lime.

Irvine considers that Hudson's method of simultaneous bromination and acetylation by a solution of hydrobromic acid in acetic anhydride is superior in many ways to the above.

Acetoiodo glucose has also been prepared. In all cases, by this method only the β derivatives are obtained. Apparently rearrangement takes place very readily during the preparation of α acetochloro

glucose by means of anhydrous hydrogen chloride and the *α* derivatives are not always obtainable; indeed, Fischer has cast some doubt on their existence.

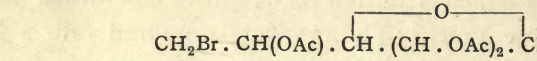
Mono-, di-, tri-, tetra- and two isomeric pentabenzoyl derivatives of glucose have been prepared. The most interesting of these is 6-monobenzoyl glucose which proves to be identical with the natural compound vacciniin isolated by Griebel from the whortleberry.

When *β*-acetobromoglucose is shaken in ethereal solution with silver carbonate and a little water tetra-acetyl glucose is obtained; this, like tetra-methyl glucose, exhibits mutarotation and exists in two forms. It yields a phenylhydrazone.

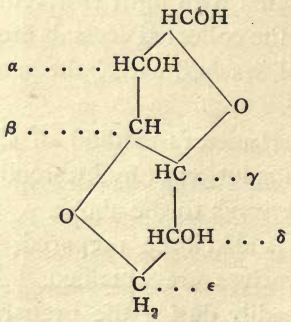
Hudson has isolated a *β*-tetra-acetyl galactose having the ethylene oxide structure which shows mutarotation with increasing dextro rotation. Acetobromo glucose also interacts with pyridine, forming tetra-acetyl glucose pyridinium bromide.

Anhydroglucose.

When the action of anhydrous hydrogen bromide on glucose pentacetate is prolonged dibromo-triacetyl glucose,



is obtained. One of the bromine atoms can be displaced by methoxyl with the formation of triacetyl methyl glucoside bromohydrin. This compound has served as the starting-point for the preparation of a new isomeride of glucosamine (p. 64). When it is heated with barium hydroxide hydrogen bromide is eliminated, and anhydromethyl glucoside, $C_7H_{12}O_5$, is formed; this when hydrolysed by dilute acids yields anhydroglucose, a well-characterised crystalline substance. It forms a phenylhydrazone and phenylosazone, both containing one molecule of water less than the corresponding glucose compounds. On the assumption of a butylene-oxide ring structure for the new anhydride, anhydro glucose will have the attached formula. This is fully in har-



mony with the deductions possible from the solid model of glucose. The ϵ-carbon being free to rotate can take up the position indicated, which is favourable for the formation of a butylene-oxide ring, linking it with the β-carbon atom through oxygen. The second bromine atom in triacetyl-dibromo glucose is in the ϵ-position, as proved by the reduction to a methylpentose (page 84).

Anhydromenthol glucoside has been obtained in a similar manner to anhydromethyl glucoside; it is of interest that emulsin is without action on either compound, though it readily hydrolyses the normal glucosides.

It would appear that the possibility of the existence in nature of anhydrides of glucose and of glucosides is not excluded, since the reduction product of anhydroglucose, anhydrosorbitol, is an isomeride of naturally occurring styracitol.

Glucal.

When β-acetobromoglucose is reduced by zinc dust and acetic acid, a peculiar compound, to which Fischer has given the name of glucal, is produced (after removal of the acetyl groups). It is a slightly sweet, soluble, viscid syrup of aldehydic properties, forming oily hydrazones but no osazones, and evidently possesses ethylenic unsaturation, since it decolorises bromine water. Fischer's later formula for glucal is :—

$$CH_2(OH).CH.CH_2.CH(OH).C:CH(OH).$$
$$\quad\quad\quad

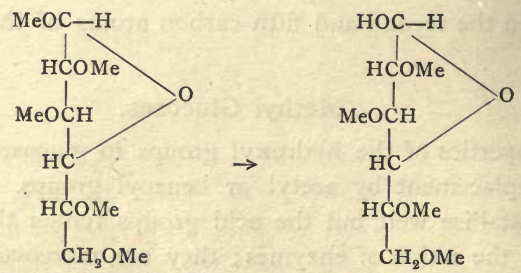
hydroxyl has a less disturbing influence; indeed, methylation has little effect on the characteristic chemical reactions of reducing sugars except in increasing stability. The reducing sugars themselves cannot be directly methylated by any of the ordinary methods; but, as Purdie and Irvine have shown, it is possible to methylate the methyl glucosides by exhaustive treatment with methyl iodide and silver oxide. The products are purified by distillation in vacuum and subsequently obtained crystalline.

This method has proved of the greatest value, since it has been found that during the reaction stereochemical changes such as racemisation, the Walden inversion, or interconversion of glucosides, do not take place. On the other hand, it is expensive, for very large excesses of the costly methyl iodide and silver oxide are necessary, and the recent work of Haworth, who has succeeded in determining the conditions under which commercial sodium hydroxide and methyl sulphate in aqueous solution may be employed without detriment to the optical purity of the products, is a welcome improvement.

Briefly, the sugar, dissolved in the least quantity of water, is stirred at a constant temperature of 70°, and in the course of an hour three times the theoretical amount of the new alkylating reagents are simultaneously added from two separate funnels; subsequently the temperature is raised to 100° for half an hour. A slight alkalinity must be maintained throughout, for even the transitory local existence of an acid system tends to induce hydrolytic changes, whilst, of course, excessive alkalinity causes enolisation, resinification, etc., to set in.

Sometimes, by this method, the last hydroxyl group to be attacked is left wholly or incompletely methylated owing to diminishing solubility of the products in the medium, and in these cases it is well to have recourse to the former method for the final stages of the methylation.

The isomeric α- and β-pentamethyl glucoses (e.g. tetramethyl-methyl glucosides), when hydrolysed by acids, are converted into tetramethyl glucoses:—



α-Pentamethyl glucose. α-Tetramethyl glucose.

Both compounds yield finally the same tetramethyl glucose of constant rotatory power, but initially *a*- and *β*-tetramethyl glucoses are obtained from them, which exhibit mutarotation and slowly change in solution into the equilibrated mixture. Tetramethyl glucose is converted by Fischer's method of etherification into a mixture of *a*- and *β*-tetramethyl-methyl glucosides.

Tetramethyl glucose is not fermentable, but tetramethyl *β*-methyl glucoside is hydrolysed by emulsin, a fact which indicates that the introduction of the methyl groups into a glucoside does not put the resulting compounds out of harmony with enzymes.

A number of other sugars have been fully alkylated in like manner.

The partially methylated derivatives of the sugar group possess a special interest, as their study has already afforded a clue to many of the vexed questions in carbohydrate chemistry. Definite mono-, di- and trimethylated hexoses have been prepared by Irvine, and their investigation has already assisted materially in the characterisation of the new ethylene oxide forms of glucose which have been described on page 13. The methods employed in their preparation consist in subjecting to methylation by the silver iodide method hexose derivatives in which certain of the hydroxyl groups are shielded from attack ; for instance, the terminal (aldehydic) hydroxyl may be transformed to methyl glucoside before the operation, or other of the hydroxyl groups may be temporarily occupied in condensation complexes with compounds such as benzaldehyde or acetone. The partially methylated glucoses are obtained on submitting these compounds to hydrolysis.

Thus, glucose diacetone forms only a monomethyl derivative, from which on hydrolysis 6-monomethylglucose,

$$CH_2(OMe).CH(OH).CH.[CH(OH)]_2.CH(OH),$$
$$\quad\quad\quad

first the benzylidene group and then the glucoside group being eliminated. Both α- and β-isomerides of the compound have been prepared; it has the constitution—

$$CH_2(OH).CH(OH).CH.[CH(OMe)]_2.CH(OH)$$
$$\quad\quad\quad

Anilides, Hydrazones, Oximes.—The interactions involved in the formation of anilides, hydrazones and oximes of glucose are most simply explained, on the assumption that the sugar is participating in a typical aldehyde reaction. None the less the occurrence of more than one form of all these derivatives forces the adoption of the closed-ring formula in such cases. Skraup early showed that a second phenylhydrazone of glucose could be isolated, isomeric with that described originally by Fischer. Isomeric benzyl phenylhydrazones have also been obtained. The rotatory power of hydrazones changes in solution. It would go too far to discuss the nature of the isomerism here, nor is it yet satisfactorily established, but it may be pointed out that glucose phenylhydrazone may be formulated in syn- and anti-forms of the true aldehydic derivative, or as α- and β-hydrazides of butylene-oxide structure, nor does this exhaust the possible isomerides.

Irvine and Moody have shown in the case of tetramethyl glucose that both the oximes and anilides possess the butylene-oxide ring in the hexose residue, and are thus to be regarded as derived from the α- or β- form of glucose, and not from an aldehydic isomeride. Their conclusions may reasonably be extended to the oximes and anilides of glucose, the latter of which Irvine and Gilmore have shown to exist in two modifications. The same authors failed to alkylate glucose phenylhydrazone or tetramethyl glucose phenylhydrazone, and consider it still an open question whether these derivatives belong to the butylene-oxide type.

The properties of a number of these derivatives are summarised in the following table :—

TABLE IV.

Glucose Derivative.	α-Series.		β-Series.	
	M.-pt.	$[\alpha]_D$.	M.-pt.	$[\alpha]_D$.
Penta-acetate	112°	+ 100°	134°	+ 3°
Acetochloro	63° (?)	—	73°	+ 165°
Acetobromo	79° (?)	—	88°	+ 198°
Acetonitro	92°	+ 1·5°	150°	+ 149°
Tetra-acetylmethyl	100°	+ 137°	105°	− 23°
Methyl glucoside	165°	+ 157°	104°	− 33°
6-Monomethyl glucose	157°	+ 96°	130°	+ 32°
2 : 3-Dimethyl glucose	85°	+ 82°	108°	+ 6°
2 : 3 : 5-Trimethyl glucose	—	—	—	—
2 : 3 : 6- „ „	—	—	—	—
3 : 5 : 6- „ „	—	—	—	—
2 : 3 : 5 : 6-Tetramethyl glucose	—	101°	—	+ 73°
Pentamethyl glucose	—	154°	—	− 17°

Stereoisomerism of the Aldohexoses.

A compound represented by the empirical formula,

$$CH_2(OH).CH(OH).CH(OH).CH(OH).CH(OH).CHO,$$

containing four asymmetric carbon atoms, should, according to the Le Bel-van't Hoff hypothesis, be capable of existing in sixteen stereoisomeric forms, eight of which would be mirror images of the other eight and of equal but opposite rotatory power.

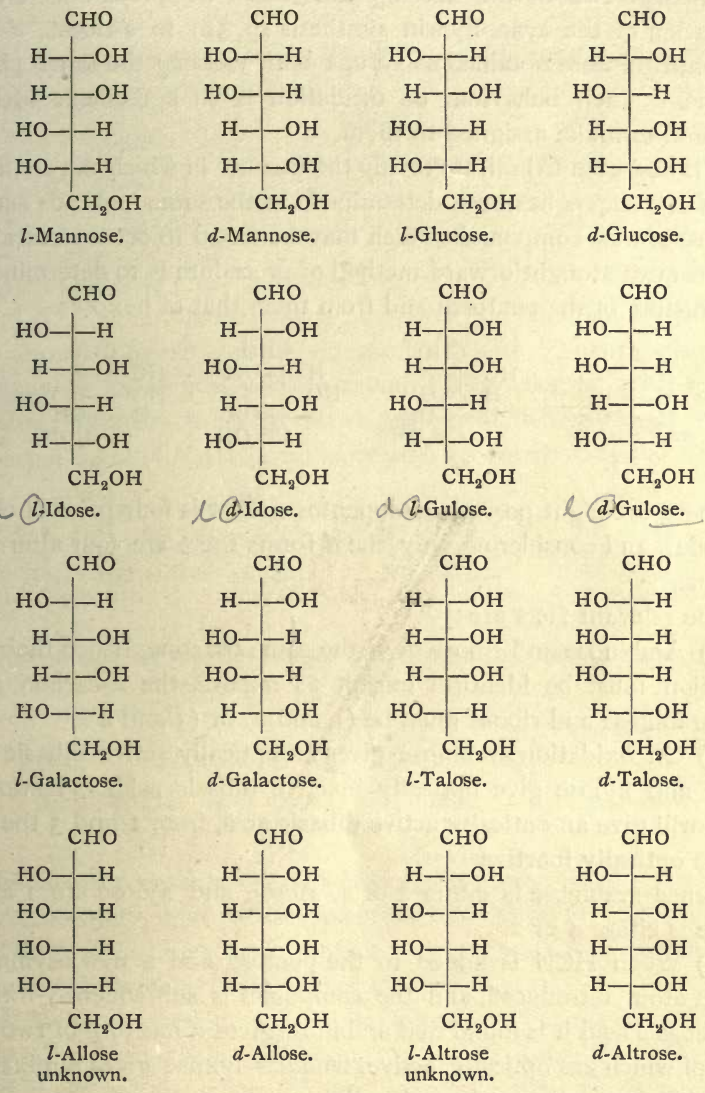
Thus, corresponding to ordinary dextro-glucose (*d*-glucose), there should be a lævo-rotatory isomeride (*l*-glucose) of equal and opposite rotatory power, of like configuration but having the dissimilar radicles in reversed order.[1] In point of fact, when glucose is prepared by artificial means from optically inactive material, a mixture in equal proportions of *d* and *l* forms is actually obtained. Such a mixture is optically inactive—whether the two forms actually combine or merely neutralise one another in optical effect is unknown.

Although only three aldohexoses occur naturally (glucose, mannose, galactose), fourteen of the sixteen possible isomerides are now known. Emil Fischer, to whom we owe the discovery of this remarkable series, has not only shown how they may be prepared, but has made them in such ways that their structural relationship may be regarded as established. His results are summarised in the following table:—

[1] The formulæ assigned to *d*- and *l*-glucose are chosen arbitrarily; that is to say, it is assumed that in the *d* form the groups occupy a certain position, whence it follows that in the stereoisomeride they are present in the reversed position. For the original proof of the validity of the formulæ and the arguments by which they are deduced, the reader is referred to Fischer's summary in the *Berichte der deutschen chemischen Gesellschaft* for 1894 (p. 3189). A further convention is to indicate as belonging to the *d*- series all compounds derived from dextro-glucose by simple reactions which leave the stereochemical structure of the molecule unchanged. In many instances, as for example *d*-fructose and *d*-arabinose, the new compound rotates polarised light to the left, so that the prefix does not give a correct indication of the sense of the rotation. Similarly all compounds derived from lævo-glucose are designated as of the *l* series though they may be dextro-rotatory. It has been possible to connect the amino acids, hydroxy acids and some other optically active substances with dextro-glucose, so that the prefix *d* has a very definite significance in these cases, the number of which is likely to increase. Unfortunately, in other cases the prefix merely denotes the sign of the rotation, so that *d*-mandelic acid, for example, which is dextro-rotatory, forms a lævo-rotatory nitrile, which is therefore termed *l*-mandelo nitrile. A new symbol other than *d* to connote relationship to *d*-glucose appears highly desirable.

TABLE V.

ALDOHEXOSES.



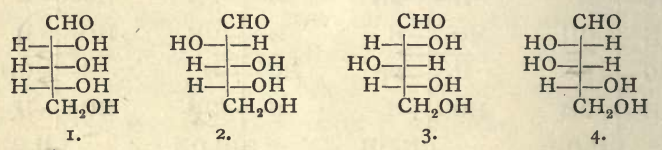
As two closed-chain butylene oxide as well as two closed-chain ethylene oxide forms should exist corresponding to each of the open chain aldehydic forms, no less a number of isomeric "glucoses" is foreseen by theory than 16 + 32 + 32 = 80.

The last four aldohexoses in the table remained unknown to Fischer, though he pointed out that they were to be derived theoretically from the isomeric riboses, only one of which had at that time been

prepared. The discovery by Levene and Jacobs that *d*-ribose is a constituent of nucleic acids, from which it can be obtained in quantity, has enabled two of the missing aldoses to be prepared. By the application of the cyanohydrin synthesis (p. 58) to *d*-ribose, *d*-allose and *d*-altrose were obtained as syrups both yielding the same phenylosazone. Their behaviour on oxidation is in agreement with the structural formulæ assigned to them.

It is desirable to indicate briefly the manner in which the configuration of the sugars has been determined, as the same methods serve in the case of new compounds which may be found to occur naturally.

The most straightforward method of procedure is to determine first the structure of the pentoses and from them that of hexoses.



There are eight possible aldopentoses, that is four pairs of optical antipodes, and considering only the *d* forms there are four alternative formulæ.

The relevant facts are :—

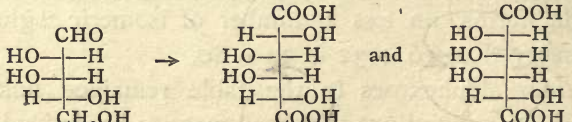
(1) Arabinose and ribose give the same osazone, hence their configuration must be identical except as regards the *a*-carbon atom. And arabinose and ribose must be (1 and 2) or (3 and 4).

(2) On oxidation arabinose gives an optically active dibasic acid; ribose and xylose give optically inactive dibasic acids. Pentoses 2 and 4 will give an optically active dibasic acid, from 1 and 3 the acids will be optically inactive.

Hence arabinose is either 2 or 4, ribose and xylose are 1 and 3, lyxose is either 4 or 2.

(3) When HCN is added to the pentose and a new asymmetric carbon atom introduced, and the compound is subsequently oxidised to a dibasic acid it is found that arabinose gives a mixture of two acids both of which are optically active, whereas lyxose gives a mixture of two acids, one active and one inactive.

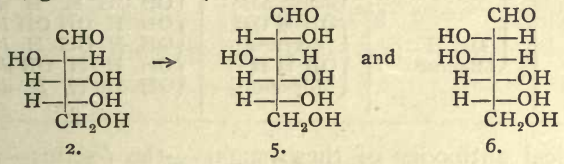
This can only happen in the case of 4 :—



Accordingly lyxose has the constitution 4 so that arabinose is 2, ribose 1, and by elimination xylose is 3.

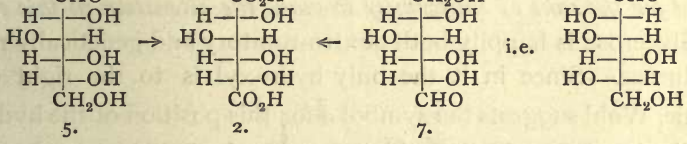
Proceeding from the pentoses to establish the formula of the hexoses we have :—

(1) Arabinose gives rise by Kiliani's reaction (addition of HCN) to two hexoses, glucose and mannose :—

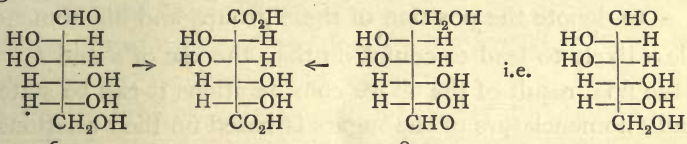


Hence glucose must be either 5 or 6.

(2) The same dibasic acid is produced on oxidation of glucose as from another hexose (gulose), viz. saccharic acid. This means that the configuration of each of the four asymmetric carbon atoms is the same and that, therefore, the difference between the two sugars is that their primary alcohol (CH_2OH) and aldehydic groups are interchanged.



In the case of 5 a new sugar 7 is formed by this process of interchange :—



In the case of 6 the same sugar 8 is formed by interchanging the groups.

Accordingly, glucose is represented by formula 5, mannose by formula 6, and gulose by formula 7. An extension of the reasoning leads to the formulæ for the other hexoses.

A useful suggestion for the simplification of the symbols showing the sterical relationships of the sugars has been made by Wohl. Instead of writing the whole formula vertically attention is confined to the H and OH groups on one side of the molecule (the right) only, and these are written down in order. If it is agreed to consider the aldehyde group as being to the right of the formula when written horizontally, *d*-glucose is OH OH H OH.

The following table shows all the possible tetrose, pentose, and hexose sugars derived from *d*-glucose, i.e. THE SUGARS OF THE *d* SERIES, according to Wohl's symbols :—

TABLE VI.

Triose.	Tetroses.	Pentoses.	Hexoses.
OH *d*-glycerose	OH OH *d*-erythrose OH H *l*-threose	OH OH OH *d*-ribose OH OH H *d*-arabinose OH H OH *l*-xylose OH H H *d*-lyxose	OH OH OH OH *d*-allose OH OH OH H *d*-altrose OH OH H OH *d*-glucose OH OH H H *d*-mannose OH H OH OH *l*-gulose OH H OH H *l*-idose OH H H OH *d*-galactose OH H H H *d*-talose

The optical antipodes of these sugars—the *l*-series—are derived from *l*-glycerose or *l*-glucose and can be incorporated in a similar table.

Wohl's work on glycerose has fortunately established that the nomenclature of the sugars as *d* and *l*, based originally by Fischer on their derivation from *d*-glucose, is equally the same when based on *d*-glycerose. Consequently, in the above table the *l* symbols of threose, xylose, gulose and idose should properly become *d*. *It is therefore proposed for the sake of uniformity to make the alteration in this edition.*

d-Glycerose is happily both dextro-rotatory and genetically related to *d*-glucose. Since in it the only hydroxyl is to the right of the molecule, Wohl suggests the symbol $\bar{d}$ for this position of the hydroxyl. Accordingly, glucose is $\overline{ddld}$ aldohexose, and symbols can be given to the sugars which avoid the use of H and OH. Fischer originally used + and − to denote the position of these groups and his nomenclature seems less likely to lead to confusion than the use of $\bar{d}$ and $\bar{l}$.

As the final result of the above considerations it can be stated that the *d* and *l* nomenclature of the sugars is based on their relationship to *d* and *l* glycerose, that is on the configuration of the fifth carbon atom, and is irrespective of the direction of their optical rotatory power.

Rotatory Powers of the α and β forms of Sugars.

Relation between Rotation and Configuration.

Most of the carbohydrates exist in more than one form and show mutarotation. Before dealing with their numeric relationships a word is required as to the nomenclature of the derivatives of sugars of the dextro and lævo series. As proposed by Hudson in the case of a dextro sugar the *α* form is that which is most dextro-rotatory whereas for a lævo sugar the reverse is the case, the more lævo-rotatory (i.e. less dextro-rotatory) modification being regarded as the *α* form. On this basis *α*-*d*-glucose becomes the optical antipode of *α*-*l*-glucose.

Ordinary crystalline maltose is thus a β-sugar; crystalline fructose is a β-sugar and not α-fructose as previously supposed. Natural xylose is considered to be genetically related to *d*-glucose and not to *l*-glucose, as supposed by Fischer.

According to van't Hoff's principle of optical superposition, as applied by Hudson, the molecular rotation of a sugar is essentially dependent on two factors: (1) the optical effect of the asymmetric system containing the reducing group, and (2) the rotatory power of the remaining asymmetric system. If these factors are represented by A and B respectively:—

$$\alpha\text{-glucose } (M)_D = 20{,}340 = +A + B,$$
$$\beta\text{-glucose } (M)_D = 3{,}420 = -A + B,$$

whence by subtraction $2A = 16{,}900$ and by addition $2B = 23{,}760$.

It is shown from the available data, firstly that the difference between the molecular rotations of the α and β forms of the aldehyde sugars and their derivatives (2A) is a nearly constant quantity, and secondly that the α and β forms of those derivatives of any aldose sugar in which only the end carbon atom is affected have molecular rotations, the sum of which (2B) is equal to the sum for the α and β forms of the aldoses.

This method enables the calculation of the rotation of the unknown isomerides of many of the sugars and their derivatives and is proving of the utmost value in elucidating questions as to their structure, as will be illustrated hereinafter.

Hudson has further shown that the mutarotating sugars have as a common property a measurable maximum rate of solution, which is caused by the slow establishment in solution of the equilibrium between the α and β forms of the sugar; those sugars, such as sucrose, trehalose, raffinose, which do not reduce Fehling's solution or show mutarotation, do not exhibit this maximum rate of solution.

Experimental evidence of the rotatory powers of those sugars for which both modifications have not yet been crystallised and measured directly has been obtained by measuring the maximum rate of solution, or the initial and final solubilities—e.g. for xylose, arabinose, lyxose, ribose, mannose, fructose, gluco-heptose, maltose, cellose.

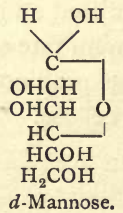
The results are summarised in the following table:—

TABLE VII.—Rotatory Powers of the Mutarotating Sugars.

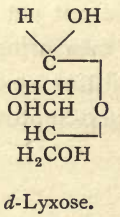
Sugar.	M. W.	Formula.	Specific Rotation in Water.			Molecular Rotation Difference.
			α Form.	Const. Rot.	β Form.	
d-Glucose	180	$C_6H_{12}O_6$	+ 113·4	+ 52·2	+ 19	+ 16,900
d-Galactose	180	$C_6H_{12}O_6$	+ 144·0	+ 80·5	+ 52	+ 16,600
d-Mannose	180	$C_6H_{12}O_6$	*+ 34*	+ 14·6	− 17	+ 9,180
d-Fructose	180	$C_6H_{12}O_6$	*− 21*	− 92·0	− 133·5	—
d-Xylose	150	$C_5H_{10}O_5$	+ 92	+ 19	*− 20*	+ 16,800
d-Lyxose	150	$C_5H_{10}O_5$	+ 5·5	− 14	*− 36*	+ 6,220
d-Arabinose	150	$C_5H_{10}O_5$	*− 54*	− 105	− 175	+ 18,100
l-Rhamnose	164	$C_6H_{12}O_5$	− 7·7	+ 8·9	*+ 54*	− 10,000
α-Glucoheptose	210	$C_7H_{14}O_7$	*+ 45*	− 20·4	− 28·4	+ 15,300
Lactose	342	$C_{12}H_{22}O_{11}$	+ 90·0	+ 55·3	+ 35	+ 18,800
Maltose	342	$C_{12}H_{22}O_{11}$	*+ 168*	+ 136	+ 118	+ 17,100
Melibiose	342	$C_{12}H_{22}O_{11}$	*+ 179*	+ 142·5	+ 124	+ 18,800
Cellose	342	$C_{12}H_{22}O_{11}$	*+ 72*	+ 35	+ 16	+ 19,200

Calculated values in italics.

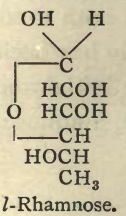
In the last column are recorded the differences between the molecular rotations of the respective alpha and beta forms of each aldose. If the rotatory power of the end asymmetric carbon atom in these aldoses has the value + A for the *α*-sugar and − A for the *β* form, and the rotation of the remainder of the structure is B, the molecular rotation of an *α*-sugar is A + B, and of its *β* form − A + B, and the difference of these values is 2A. It is to be expected, on the view that the value of A is not influenced by changes in the configuration of the remainder of the molecule, that this difference 2A is a constant for all the aldoses. The last column shows that the theory is fairly well borne out except in the case of mannose, lyxose and rhamnose. Fructose is not considered, since it is a ketose and does not apply in the theory. The negative sign for the difference in the case of rhamnose is the result of the system of nomenclature for the *α* and *β* forms and is due to the fact that rhamnose is an *l*-series sugar. Now the configurations of *d*-mannose, *d*-lyxose and *l*-rhamnose are



d-Mannose.



d-Lyxose.



l-Rhamnose.

and it will be observed that these configurations are identical (or antipodal) from the γ-carbon atom upward. It appears probable, therefore, that the exceptional value of the difference for these sugars may be dependent upon this type of configuration. Since, however, *α*-glucoheptose

has the same configuration from the γ-carbon upward, and nevertheless shows a molecular difference nearer, though not equal, to the average value for most of the aldoses, this possible connection between structure and exceptional rotation remains in some doubt. In the case of closely related sugars, such as the four disaccharides, the agreement between theory and experiment is very good when it is recalled that it has been possible to make the measurements only by an indirect procedure.

A comparison of the formulæ of glucose, mannose, xylose, lyxose, which differ only in the configuration of the α-carbon atom and their molecular rotations has enabled Hudson to deduce the value of the molecular rotation of this carbon as + 4,500 for glucose and xylose and − 4,500 for mannose and lyxose.

The glycol glucosides may be quoted as examples of Hudson's second rule:—

	$(M)_D$.	
α-Glucose	20,340 =	A + B
β-Glucose	3,420 =	−A + B
Sum	23,760 =	2B
Glycol-α-glucoside	30,347 =	A′ + B
Glycol-β-glucoside	6,843 =	−A′ + B
Sum	23,504 =	2B

whilst the monomethylglucoses afford a further example of the first rule:—

Monomethyl-α-glucose	20,874 =	A + B′
Monomethyl-β-glucose	4,733 =	−A + B′
Difference	16,141 =	2A

For glucose the difference is 16,900.

The relative ease with which the isomeric fully acetylated derivatives are prepared and purified makes them especially suitable for testing the relation of rotation and constitution. The difference in the molecular rotations of the α- and β-sugars should be a constant as is evidenced by the following figures:—

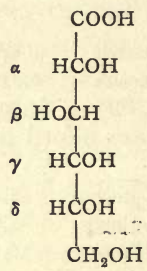
TABLE VIII.

Substance.	Molecular Rotation of α form.	Molecular Rotation of β form.	Difference.
d-Glucose pentacetate . . .	+ 39,600	+ 1,500	+ 38,100
d-Lactose octacetate . . .	+ 36,500	− 2,900	+ 39,400
d-Maltose octacetate . . .	+ 83,000	+ 42,500	+ 40,500
d-Cellose octacetate . . .	+ 27,800	− 10,200	+ 38,000
d-Glucosamine pentacetate . .	+ 36,400	+ 470	+ 35,930
d-Chondrosamine pentacetate . .	+ 39,500	+ 4,100	+ 35,400
d-Gentiobiose octacetate . .	+ 35,500	− 3,600	+ 39,100
d-α-Glucoheptose hexacetate . .	+ 40,200	+ 2,200	+ 38,000
d-Mannose pentacetate . . .	+ 21,400	− 9,800	+ 31,200
d-Galactose pentacetate . . .	+ 41,600	+ 8,900	+ 32,700
d-Xylose tetracetate . . .	+ 28,300	− 7,900	+ 36,200
l-Arabinose tetracetate . . .	+ 13,400	+ 46,800	− 33,400

The sign and magnitude of the optical rotatory power enable a considerable insight to be gained into the structure of the molecule. For example, it is established in the case of twenty-four lactones of the monobasic sugar acids and eleven lactones of the saccharinic acids that polarised light is rotated to the right or to the left according as the butylene oxide ring on the γ-carbon is on the right or left of the configuration (when the formula is written with the acid group on top, as on p. 54). Accordingly, the configuration of the γ-carbon atom of any new lactone can at once be determined from its rotation.

Similar deductions as to the configuration of the α-carbon atom may be made from the direction of rotation of the phenylhydrazide of the acid. If the phenylhydrazide rotates to the right the hydroxyl on the α-carbon atom is on the right, and vice versa.

In gluconic acid, for example, there are four asymmetric carbon atoms, α, β, γ, δ, and the molecular rotation may be expressed as $+\alpha$, $-\beta$, $+\gamma$, $+\delta$, the + value indicating an hydroxyl on the right.



Hudson has deduced values for α, β, γ and δ, by solving the four equations for the phenylhydrazides of gluconic, gulonic, idonic and galactonic acids, for which the rotations have been measured by Nef. He finds the comparative values ($\times 10^2$) are—

$$\alpha + 37{\cdot}3^\circ, \beta + 3{\cdot}9^\circ, \gamma + 1{\cdot}4^\circ, \delta - 0{\cdot}6^\circ,$$

showing that the value of α, the rotation of the α-carbon atom is so very much larger than the values of the rotations of the other three carbons that its sign determines the direction of the rotation.

The same rule holds good in the case of the amides of these acids, of which Weerman has measured the rotatory power. The specific rotations are small so that the calculation is only approximate, but it yields the following figures—

$$\alpha + 32^\circ, \beta - 10^\circ, \gamma - 1^\circ, \delta - 7^\circ,$$

which show once again that the direction of rotation is influenced by the α-carbon atom.

Levene finds for the salts of the monobasic acids that the con-

figuration of the α-carbon atom has a strong influence on the rotation. The values calculated from his observations are—

$$\alpha + 22°, \beta + 13°, \gamma + 12°, \delta - 4°.$$

These are quite different from those found for the phenylhydrazides and amides, owing perhaps to the salts being largely dissociated. It is evident further that the influence of the α-carbon is less than that of the sum of the other three carbons.

Lastly, it may be mentioned that the benzylphenylhydrazones of the sugars rotate to the left when the asymmetric α-carbon atom of the configuration has its hydroxyl to the right, and vice versa.

Enough has been said to show the large amount of certainty with which a part of the configuration formula of a sugar may be deduced from the optical rotation of its derivatives, and it is to be expected that the extension of these methods of investigation will go far to clear up the outstanding problems of structure both for the carbohydrates and other aliphatic hydroxy compounds.

CHAPTER II.

THE CHEMICAL PROPERTIES OF GLUCOSE AND THE HEXOSES.

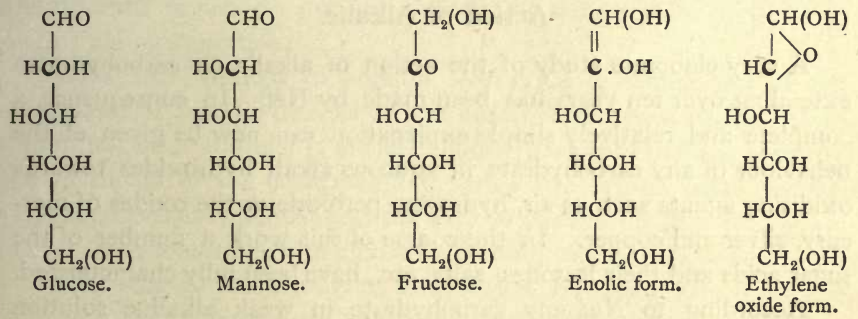
GLUCOSE, the other aldoses and the ketoses in general show a great tendency to become further oxidised; this is evidenced by their activity as reducing agents. They reduce alkaline copper solutions on warming, forming red cuprous oxide, likewise ammoniacal silver solutions forming a metallic mirror. When heated with alkali, a sugar solution colours at first yellow, subsequently brown and finally decomposes; a variety of substances, including lactic acid and other hydroxy acids, are formed. Valuable analytical methods for the estimation of glucose are based on the reaction with copper salts in alkaline solution, but the precise changes which the sugar undergoes under these conditions are not completely understood.

When carbohydrates are kept with alkali hydroxide at 37° the optical rotation of the solution decreases and the acidity increases. Sodium hydroxide exerts the greatest action, sodium carbonate being considerably weaker; ammonia of the same strength is almost without action.

The ketose sugars without exception are decomposed when their aqueous solutions are exposed in quartz tubes to sunlight. Carbon monoxide is evolved and the corresponding alcohol containing one carbon atom less is formed. The aldose sugars are practically unaffected under these conditions. Exposure of the ketoses to the ultra violet light from a mercury lamp brings about the same decomposition, but other actions also take place involving the formation of hydrogen, methane, formaldehyde and non-volatile acids. The aldoses are decomposed in a similar manner to the ketoses by ultra violet rays but are less susceptible to attack.

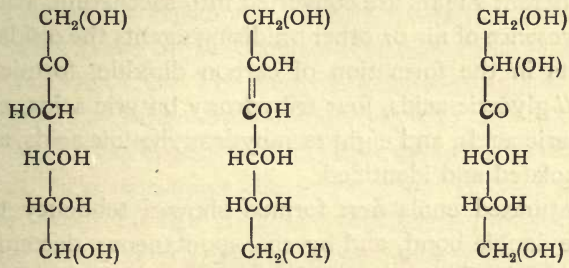
Interconversion of Glucose, Fructose and Mannose.

Glucose, fructose and mannose pass over into one another in aqueous solution in presence of alkalis. This most important transformation was first observed by Lobry de Bruyn and Van Ekenstein; it takes place slowly at ordinary temperatures, quickly and with much decomposition at higher temperatures. Starting from glucose, the optical rotation is observed to fall to about 0°; considerably more fructose than mannose is formed in the final product. The change was rightly explained by Wohl as due to conversion into the enolic (unsaturated) form common to all three carbohydrates:—



The sugar originally present is slowly transformed into enol; this is reconverted into all three of the possible hexoses. It is to be supposed that the formation of enol from each one of the hexoses and the reverse changes all take place with different velocities; the interchange is further complicated by secondary effects.

For example, fructose can give rise to a second enolic form, and this will occasion the formation of other isomerides, e.g. glutose:—



which Lobry de Bruyn has isolated as a regular product of the transformation of glucose. The change is obviously exceedingly complicated. Prolonged action of the alkali or action at a high temperature leads to the formation of hydroxy acids. In pure aqueous solution glucose can

be kept for years without alteration. This proves that there can be no enolic form present in the equilibrated mixture of *a*- and *β*-glucose as is sometimes suggested. It is highly probable that the ethylene oxide form, rather than the unsaturated enolic form, is actually present in solution.

The guanidine compounds of glucose, fructose and mannose show changes of rotatory power in aqueous solution due to the interconversion of the three hexoses brought about by the guanidine. The changes are very similar to those caused by alkalis, but fewer secondary changes take place in the case of guanidine.

Action of Alkalis.

A very elaborate study of the action of alkalis on carbohydrates extending over ten years has been made by Nef. In consequence, a complete and relatively simple explanation can now be given of the behaviour of any carbohydrate in aqueous alkali hydroxides towards oxidising agents such as air, hydrogen peroxide or the oxides of mercury, silver and copper. In the course of this work a number of the sugar acids and their lactones, salts, etc., have been fully characterized.

According to Nef any carbohydrate in weak alkaline solution undergoes profound change but is eventually transformed into an equilibrated mixture in which no less than one hundred and sixteen substances can in theory take part. These are the thirty-two aldoses with one to six carbon atoms, the thirty-two corresponding methylenols, the twenty-six ketoses with three to six carbon atoms in an unbranched chain and the twenty-six dienols. Actually in practice only ninety-three different substances are formed, and in the absence of an oxidising agent the different sugars are converted into saccharinic acids.

In the presence of air or other oxidising agents the oxidation of the sugars results in the formation of carbon dioxide, formic, glycollic, oxalic and *dl*-glyceric acids, four trihydroxy butyric acids, eight tetrahydroxy valeric acids, and eight tetrahydroxyhexoic acids, all of which have been isolated and identified.

The unsaturated enols first formed show a tendency to undergo fission at the double bond, and by the spontaneous decomposition of the *aβ*-, *βγ*- and *γδ*-dienols, any hexose may yield (*a*) formaldehyde and aldopentoses, (*b*) diose and aldotetroses, and (*c*) *dl*-glyceraldehyde.

Taking glucose as a type the following products result :—

(*a*) From glucose *aβ*-dienol → formaldehyde and arabinose.
(*b*) From glucose *βγ*-dienol → diose and triose.
(*c*) From glucose *γδ*-dienol → glyceraldehyde.

An example of transformation (*a*) which has been the most difficult to verify experimentally, is furnished by the formation of *d*-arabonic acid on oxidation of glucose in weak alkaline solution by air.

When galactose is oxidised by a stream of air at 30°-40° in presence of six equivalents of sodium hydroxide the products from 50 grammes of sugar were 1·27 grammes of carbon dioxide, 12·8 grammes of formic acid and 41 grammes of non-volatile hydroxy acids. When hydrogen peroxide is the oxidising agent the quantity of the C_5 acids is less but still exceeds the amount of the C_6 acids, whereas with Fehling's solution the reverse is the case. A resin is formed in varying amounts during oxidation; it is considered that this is the explanation why the different sugars do not give exactly the same results by titration with Fehling's solution.

When the amount of sodium hydroxide is diminished to 0·5 equivalent or less the number of substances in the system after equilibrium has been attained is much smaller—thus only the six isomeric active sugars of the corresponding series are formed from glucose. The equilibrium is limited and the various dienols do not decompose under these conditions into aldoses according to fission (*a*) above.

The relative quantities of the sugars obtained are strikingly different, whilst ketoses are only enolised in quite definite directions, i.e. only certain preferred olefine dienols are formed and not all those theoretically possible. For example, from glucose and $\frac{1}{20}$ equivalent of calcium hydroxide, aldoses and ketoses are formed in approximately equal quantities, whilst the aldoses consist of glucose and mannose in the ratio of 5 : 1. In the case of galactose only the $a\beta$- and $\beta\gamma$-dienols are formed and galactose comprises 90 per cent. of the aldoses. Arabinose or xylose yield the corresponding three active pentoses.

In the case of all carbohydrates salt formation with the alkali hydroxide takes place at the carbon atom next the carbonyl group CH(OH) . CH(OM) . CO. The methylene derivative CH(OH) . $\overset{\wedge}{\underset{\wedge}{C}}$. CO forms first glycide and then *ortho*-osone, CH_2 . CO . CO, from which by the benzilic acid transformation saccharinic acids are formed. In the presence of an oxidising agent the 1 : 2 osone, CH(OH) . CO . CO, is formed. To avoid further changes *ortho*-osone formation must be effected in neutral or faintly acid solution, the best reagent being lead hydroxide or chloride or basic acetate.

Both resins and polysaccharides are formed when a sugar solution is kept or warmed in contact with very dilute alkali hydroxide or carbonate. The polysaccharides synthesized belong to two classes according to the ease with which they are hydrolysed by acids.

Nef's investigations give an explanation of the quantitative differences in the behaviour of the various sugars towards Fehling's solution.

Saccharinic Acids.

The saccharinic acids are formed from the hexoses by the action of concentrated alkali hydroxide. Twenty-four isomeric acids with six carbon atoms are theoretically possible, viz. :—

(1) Eight stereoisomeric metasaccharinic acids,

$$CH_2(OH) . CH(OH) . CH(OH) . CH_2 . CH(OH) . CO_2H$$

derived from the sixteen aldohexoses.

(2) Four isosaccharinic acids derived from the eight ketoses,

$$CH_2(OH) . CH(OH) . CH_2 . C(OH)\begin{matrix}CO_2H \\ CH_2OH\end{matrix}$$

(3) Eight saccharinic acids—

$$CH_2(OH) . CH(OH) . CH(OH) . C(OH)\begin{matrix}CO_2H \\ CH_3\end{matrix}$$

(4) Four parasaccharinic acids—

$$CH_2(OH) . CH(OH) . C(OH)\begin{matrix}CO_2H \\ CH_2 . CH_2(OH)\end{matrix}$$

The lactones of these acids are termed saccharins. Nef has very carefully studied these substances: their fuller treatment lies outside the scope of this monograph.

Since lactic acid and various hydroxy acids result from the action of alkalis on glucose, the action of ammonia might cause the formation of alanine or other amino acids. Windaus and Knoop, in investigating this point, find that the strongly dissociated zinc hydroxide ammonia acts on glucose even in the cold, producing methyl glyoxaline, a closed-ring compound containing nitrogen. Amino acids are not formed. To explain this transformation, it is assumed that glyceric aldehyde is first formed, which passes into methyl glyoxal; this in its turn is acted upon by ammonia and formaldehyde to give methyl glyoxaline :—

$$CH_3 . CO . CHO + 2NH_3 + HCHO = \begin{matrix}CH_3 . C . NH \\ \| \quad\quad \diagdown \\ CH . N \diagup\!\!\diagup CH\end{matrix}$$

Windaus finds that the reaction is not confined to glucose, but that the same methyl glyoxaline is yielded by mannose, fructose, sorbose, arabinose, xylose and rhamnose, or by the disaccharide lactose.

The formation of the glyoxaline nucleus from the sugars is of considerable interest in view of the important place this holds among natural products. Thus it is present in ergot, in pilocarpine and in

the purines. Further, by condensation of methylglyoxaline with glycine and simultaneous oxidation, histidine is formed.

Interaction with Phenyl Hydrazine.

Particularly characteristic is the behaviour of the sugars with excess of phenyl hydrazine on heating in dilute acetic acid solution. An orange-yellow insoluble phenyl osazone is formed, which serves to characterise glucose even when present only in very small quantities, though not to distinguish it from some of the isomeric hexoses which give the same or closely related phenyl osazones. The use of phenyl hydrazine possesses further a historical interest, as in the hands of Emil Fischer it served as one of the chief aids in the elucidation of the chemistry of the carbohydrates.

Glucose and phenyl hydrazine interact in acid solution, acetic acid being usually employed, in two stages. In the first, which takes place in cold solution, a phenyl hydrazone is formed :—

$$C_6H_{12}O_6 + C_6H_5 . NH . NH_2 = C_5H_{11}O_5 . CH : N . NH . C_6H_5 + H_2O$$

This is a colourless compound, soluble in water, existing in two modifications, one or other of which is obtained according to the method of preparation.

Skraup's β-phenyl hydrazone, formed by shaking glucose with phenyl hydrazine in alcoholic solution, crystallises in needles, m.p. 106°-107°, and has an optical rotation in aqueous solution of $[\alpha]_D - 2°$ changing to $- 50°$. Fischer's α-isomeride, formed in alcoholic acetic acid solution, crystallises in leaflets, m.p. 159°-160°, $[\alpha]_D - 70°$ changing to $- 50°$. Behrend has shown Skraup's β-isomeride to be in reality a compound of phenyl hydrazine (1 mol.) with 2 molecules of the β-hydrazone. This hydrazone also forms an additive compound with pyridine which, on treatment with alcohol, yields glucose β-phenyl hydrazone, m.p. 140°-141°, $[\alpha]_D - 5{\cdot}5°$. Behrend has advanced evidence to show that this is a true hydrazone,

$$CH_2(OH) . [CH(OH)]_4 . CH : N . NHPh,$$

whereas Fischer's glucose α-phenyl hydrazone is a hydrazide :—

$$CH_2(OH) . CH(OH) . \overset{\overset{\text{———O———}}{|\qquad\qquad|}}{CH} . [CH(OH)]_2 . CH . NH . NHPh$$

It should be capable of existing in two stereoisomeric forms (cp. p. 33).

The phenyl hydrazones of glucose and most of the other sugars, being easily soluble, are not adapted for characterising the parent sugars. An exception is afforded by mannose, which forms an almost insoluble

phenyl hydrazone and can thus be very readily detected. This compound affords a striking illustration of the influence exercised by the configuration of the molecule on its physical properties. Sparingly soluble phenyl hydrazones are also formed by the methyl pentoses.

Asymmetrically disubstituted hydrazines of the type, $NH_2 . NR . C_6H_5$, such as methylphenyl, benzylphenyl or diphenyl hydrazines, also react with the sugars, and some of these hydrazones are sparingly soluble and are characteristic of a particular sugar. Many of them are included in the following Table IX. In some instances two forms of the hydrazone have been described.

Thus the methylphenyl hydrazone is characteristic of galactose and the diphenyl hydrazone of arabinose. The influence of the position of the OH groups on the physical properties is even more marked in the case of the dihydrazones formed with diphenylmethane dimethyl dihydrazine, $CH_2[C_6H_4NMe . NH_2]_2$ (Braun). Hydrazones are only produced when at least two or three of the hydroxyl groups attached to the carbon atoms immediately adjacent to the aldehydic group have the same spatial configuration. Thus, rhodeose, fucose, mannose, galactose, ribose, lyxose, arabinose, and rhamnose give hydrazones with this reagent, whilst isorhodeose, glucose, and xylose do not. It is suggested that the reagent may be useful in deciding questions of configuration of the aldoses in view of this peculiarity.

TABLE IX.—Melting-points of Sugar Hydrazones and Osazones.

	Arabinose.	Glucose.	Mannose.	Galactose.	Maltose.	Lactose.
Hydrazones.						
Phenyl hydrazone	151°-153°	{115°-116° / 144°-146°}	186°-188°	158°	—	—
p-Bromophenyl hydrazone	150°	147°	208°-210°	168°	—	—
α-Methylphenyl hydrazone	161°	130°	178°	180°	—	—
α-Ethylphenyl hydrazone	153°	—	159°	169°	—	—
α-Amylphenyl hydrazone	120°	128°	134°	116°	—	123°
α-Allylphenyl hydrazone	145°	155°	142°	157°	—	132°
α-Benzoylphenyl hydrazone	170°	165°	165°	154°	—	128°
Diphenyl hydrazone	218°	161°	155°	157°	—	—
β-Naphthyl hydrazone	141°	—	157°	167°	176°	203°
Osazones.						
Phenyl osazone	160°	208°	208°	193°	206°	200°
p-Bromophenyl osazone	196°-200°	222°	—	—	198°	—
p-Nitrophenyl osazone	—	257°	—	—	261°	258°

To prepare the phenyl osazone, glucose is heated with a considerable excess of phenyl hydrazine[1] (3-4 mols.) and acetic acid, the vessel being

[1] It is important that the phenyl hydrazine should be almost colourless and free from oxidation products.

immersed in rapidly boiling water for an hour or more, when the insoluble osazone separates: it is best purified by crystallisation from a dilute solution of pyridine. The excess of phenyl hydrazine acts as an oxidising agent towards the phenyl hydrazone, converting the penultimate – CH(OH) group into – CO and being itself reduced to aniline and ammonia. The CO group so formed interacts with a further molecule of phenyl hydrazine to form the osazone:—

CHO	CH : N . NHPh	CH : N . NHPh	CH : N . NHPh
CH(OH)	CH(OH)	CO	C : N . NHPh
CH(OH)	CH(OH)	CH(OH)	CH(OH)
CH(OH)	CH(OH)	CH(OH)	CH(OH)
CH(OH)	CH(OH)	CH(OH)	CH(OH)
$CH_2(OH)$	$CH_2(OH)$	$CH_2(OH)$	$CH_2(OH)$
Aldose →	Hydrazone →	Oxidation product →	Osazone

Glucose, mannose and fructose yield the same phenyl osazone, since the asymmetry of the α-carbon atom is destroyed in its formation. The osazones of the different sugars are as a class very similar in properties, those formed by the disaccharides being distinguished by their greater solubility in boiling water. The melting-points of the osazones depend very largely on the rate of heating and on the method of purification adopted, and too much dependence is not to be placed on them in identifying unknown sugars. Fischer, for example, states that carefully purified glucosazone heated rapidly in a narrow capillary tube begins to melt at 208° (corrected), and completely melts at this temperature with decomposition if the source of heat be withdrawn. When heating is continued at the same rate the thermometer rises to 213° before the glucosazone completely melts. When the heating is slower the substance begins to sinter and melt at 195°. In the case of the disaccharides, where the purification of the osazone is more difficult, the determination of the exact melting-point is even less reliable.

The asymmetrically disubstituted hydrazines do not form osazones with glucose on account of their being unable to act as oxidising agents. Fructose is more easily attacked by them, probably in consequence of the presence of the $CH_2(OH).CO$ group, and yields a methylphenyl osazone.

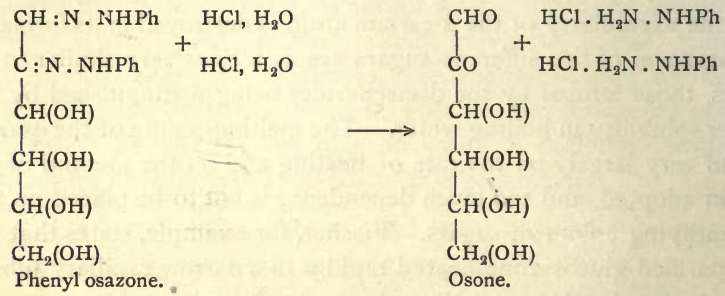
It is often a matter of considerable difficulty to obtain a carbohydrate in a pure state from solutions which may also contain inorganic salts or nitrogenous substances. One of the methods adopted is to isolate the phenyl hydrazone, purify this by crystallisation, and decompose it into sugar and phenyl hydrazine. Fischer originally used fuming

hydrochloric acid to effect the decomposition. Benzaldehyde was substituted for this by Herzfeld; the phenyl hydrazone is boiled in water with a slight excess of benzaldehyde, and the phenyl hydrazine removed from solution as insoluble benzaldehyde phenyl hydrazone,

$$C_6H_{12}O_5 : N . NHPh + C_6H_5 . CHO = C_6H_{12}O_6 + C_6H_5CH : N . NHPh.$$

This method was repeatedly adopted with success by Fischer, but it gives less satisfactory results with the disubstituted hydrazones, in which case formaldehyde may with advantage be substituted for benzaldehyde, as suggested by Ruff and Ollendorf. The hydrazone is dissolved in dilute formaldehyde and heated at the temperature of the water bath, $C_6H_{12}O_5 : N . NRR' + HCHO = C_6H_{12}O_6 + H . CH : N . NRR'$. The excess of formaldehyde is removed and the pure sugar solution concentrated in a vacuum.

Fuming hydrochloric acid acts on the osazone in the same manner as it does on the hydrazone, eliminating in this instance both hydrazine groups to form an osone :—



Phenyl osazone. Osone.

Glucose, mannose and fructose, which form the same phenyl osazone, likewise form the same osone. These osones are colourless syrups; they act as strong reducing agents, and combine directly with phenyl hydrazine or with disubstituted phenyl hydrazines forming osazones. The osones combine also with *o*-phenylene diamine. They are not fermentable. On reduction glucosone is converted into fructose. This is the only method available of regenerating a sugar from the phenyl osazone. When the sugar originally used was an aldose the corresponding ketose results. The method is of great historical interest, as by its aid Fischer established the nature of the synthetical *a*-acrose. The osazones of the disaccharides are hydrolysed by acids to hexose, hexosone and phenylhydrazine—

$$C_6H_{11}O_5 . O . C_6H_{10}O_4(N_2HPh)_2 + 2HCl + 3H_2O$$
$$= C_6H_{12}O_6 + C_6H_{10}O_6 + 2NH_2 . NHPh . HCl$$
Hexose. Hexosone.

—and Fischer's hydrochloric acid method is thus not available for the conversion into osone. Since, however, the osazones of the disac-

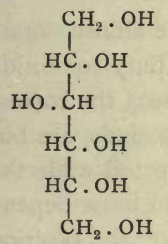
charides are soluble in boiling water, it is possible to remove the phenyl hydrazine residues by means of benzaldehyde (Fischer and Armstrong), and so obtain the osones—

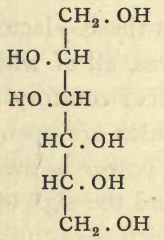
$$C_6H_{11}O_5 . O . CH_2 . [CH . OH]_3 . CO . CHO$$

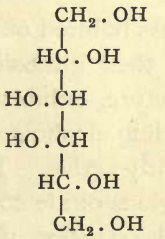
These osones are similar to glucosone in properties: they are hydrolysed by enzymes in the same way as the parent disaccharides.

Reduction.

When reduced with sodium amalgam, glucose and its isomerides form the corresponding hexahydric alcohols, two hydrogen atoms being added to the hexose. Sorbitol is formed from glucose, mannitol from mannose, and dulcitol from galactose. Fructose yields a mixture of the two alcohols, sorbitol and mannitol, since a new asymmetric carbon atom is formed from the ketonic radicle. These alcohols have the following configuration formulæ:—


Sorbitol.


Mannitol.


Dulcitol.

All three alcohols occur in plants, mannitol being widely distributed. In the fungi and some other orders mannitol exceeds glucose in quantity, or even replaces it. None of the alcohols are fermented by yeasts; mannitol, however, is a product of some bacterial fermentations, and is attacked by many moulds and bacteria. Dulcitol, no doubt on account of the difference in configuration, is in general far more resistant to bacterial attack.

All these alcohols are sweet, well-crystallised compounds quite soluble in water and alcohol. They form hexacetyl, hexabenzoyl, and explosive hexanitro derivatives, also compounds with acetone and benzaldehyde.

Their configuration is discussed in detail in the next chapter.

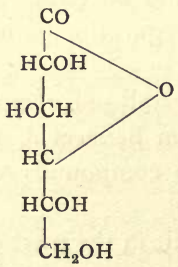
Oxidation.

Glucose on oxidation gives rise to three acids containing the same number of carbon atoms; two of these acids are monobasic, the third is dibasic. Their structure is as follows:—

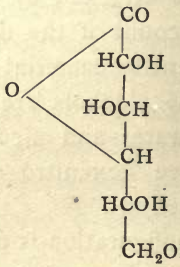
CHO	CO_2H	CHO	CO_2H
$(\dot{C}H \cdot OH)_4$	$(\dot{C}H \cdot OH)_4$	$(\dot{C}H \cdot OH)_4$	$(\dot{C}H \cdot OH)_4$
$\dot{C}H_2OH$	$\dot{C}H_2OH$	$\dot{C}O_2H$	$\dot{C}O_2H$
Glucose.	Gluconic acid.	Glucuronic acid.	Saccharic acid.

In **gluconic acid** the aldehyde group of glucose is oxidised to carboxyl: it is conveniently prepared by the action of bromine on glucose. Gluconic acid in solution very readily passes over into a γ-lactone, the change, which is accompanied by an alteration in rotatory power, being a reversible one. The reaction is not complete, but continues until an equilibrium between acid and lactone is reached. Mannose and other aldoses form mannonic acid and similar acids corresponding to gluconic acid.

As pointed out by Hudson these γ-lactones, like the aldose sugars and their glucosidic derivatives, all of which have a butylene-oxide structure, exhibit strong optical rotatory power, whereas the corresponding alcohols and acids, which are open-chain compounds, are but slightly active. The rotatory power is evidently connected with the butylene-oxide constitution, and the sign of the rotation must depend on the position of the ring, which is in turn dependent on the position of the hydroxyl group attached to the γ-carbon atom before the ring was produced. According to Hudson the dextro-rotatory lactones have the ring on one side of the structure, whilst the lævo-rotatory lactones have rings on the other side as is illustrated by the lactones of gluconic and galactonic acids.



Gluconic lactone.
$[\alpha]_D + 68°$.



Galactonic lactone.
$[\alpha]_D - 7{\cdot}07°$.

The theory has been extended to the determination of the constitution of lactones of unknown structure. It does not apply to the aldoses themselves or to the glucosides.

Whereas most of the monobasic sugar acids form γ-lactones, *d*-mannonic acid is remarkable in that when liberated from its salts at the ordinary temperature it changes almost entirely into a β-lactone.

$$CH_2(OH).(CH.OH)_2.\overset{\text{———O———}}{\underset{\text{|}\qquad\qquad\text{|}}{CH.CH(OH).CO}}$$

To obtain the γ-lactone it is necessary to evaporate a solution of the acid or of the β-lactone to dryness, preferably in presence of a little hydrochloric acid. The β-lactone rapidly undergoes spontaneous conversion into the parent acid in aqueous solution and is much less stable than the γ-lactone. Similarly, gluconic acid yields both a β- and a γ-lactone, and Nef claims that bimolecular α-lactones are also formed by the hydroxy acids.

The behaviour of the methylated mannonic acids lends no support to the idea that α-lactones are readily formed. Thus Irvine and Paterson have shown that the acid

$$CH_2(OMe).CH(OMe).CH(OH).[CH.OMe]_2.CO_2H$$

forms a lactone whereas

$$CH_2(OMe).(CH.OMe)_3.CH(OH).CO_2H$$

could not be converted into any such derivative.

The rate of action of bromine water on the aldoses is influenced considerably by their configuration: galactose, for example, is much more rapidly oxidised than glucose. (Votoček and Němeček.)

An important property of gluconic and similar acids, and one which has been of the utmost value in effecting the synthesis of the sugars, is their behaviour on heating with quinoline or pyridine. It is well known that in most substances containing an asymmetric carbon atom, rearrangement takes place, when they are heated, so as to form the corresponding antimere mixed with the original substance. When gluconic acid is heated with quinoline or pyridine at 130°-150° it is partially converted into mannonic acid. The rearrangement is apparently restricted to the groups attached to the α-carbon atom, as is the case in the transformation of glucose to mannose by alkalis. It is reversible, mannonic acid being converted into gluconic acid:—

CO_2H		CO_2H
$H.\dot{C}.OH$	$\rightleftharpoons$	$HO.\dot{C}.H$
$(\dot{C}H.OH)_3$		$(\dot{C}H.OH)_3$
$\dot{C}H_2OH$		$\dot{C}H_2OH$
d-Gluconic acid.		*d*-Mannonic acid.

Similarly, *d*-galactonic and *d*-talonic acid are mutually interconvertible.

Saccharic acid is formed by the action of nitric acid on glucose; it forms a sparingly soluble acid potassium salt, which serves as a

test for glucose. Saccharic acid is also produced from sucrose, raffinose, trehalose, dextrin and starch, all of which contain glucose. On the other hand, **mucic acid**—the corresponding oxidation product of galactose—is produced by the action of nitric acid on galactose, dulcitol, lactose, melibiose and the gums.

Free saccharic acid is crystalline; in solution it passes into the monobasic γ-lactonic acid until equilibrium is attained, the rotatory power rising from 9·7° to 22·5°. The lactone crystallises in leaflets: in solution the rotation falls from 38° to 22° as it is converted into the free acid.

Mucic acid has a sandy crystalline structure and is optically inactive. When distilled alone pyromucic acid (furfurane-*a*-carboxylic acid) is formed. Heating with hydrobromic acid converts it into dehydromucic acid (furfurane-*aa*-di-carboxylic acid).

Glucuronic Acid.[1]—Physiologically the most interesting oxidation product of glucose is glucuronic acid, which is frequently found in the urine, combined with a variety of substances, forming compounds of glucosidic nature. Normally glucose is rapidly oxidised in the animal organism to carbon dioxide and water. When certain substances, such as chloral or camphor, which are oxidised in the body only with difficulty, are brought into the system, the organism has the power of combining them with glucose to form glucosides. In such compounds one end of the glucose molecule is shielded from attack, but oxidation takes place at the other extremity of the molecule, and a glucuronic acid derivative is formed. They are excreted in the urine. The faculty of removing injurious substances from circulation in combination with glucose seems to be common to both the animal and the vegetable kingdom, and the glucosides in the plant may be compared to the glucuronic acid derivatives in the animal. The glucuronates behave like glucosides, and form glucuronic acid when hydrolysed by mineral acids. The glucuronate most commonly employed for the preparation of the acid is euxanthic acid, a substance obtained in India from the urine of cows which have been fed with mango leaves. Euxanthic acid is very readily hydrolysed by dilute acids and breaks down into euxanthon, i.e. 2 : 8-hydroxyxanthone and glucuronic acid—

$$C_{19}H_{18}O_{11} = C_{13}H_8O_4 + C_6H_{10}O_7$$

A vast number of substances when introduced into the organism are excreted in the urine as "paired" glucuronic acid compounds. Almost every organic group yields an example. The most important are included in the following list:—

[1] Also written Glycuronic acid.

isopropyl alcohol	chloral	benzene	turpentine oil
methylpropyl carbinol	butylchloral	nitrobenzene	camphor
methylhexyl carbinol	bromal	aniline	borneol
tertiary butyl alcohol	dichloracetone	phenol	menthol
tertiary amyl alcohol		resorcinol	pinene
pinacone		thymol	antipyrine
		α- and β-naphthol	etc.

As the formula indicates, glucuronic acid is the first reduction product of saccharic acid, and it was obtained in this way by Fischer and Piloty from saccharic acid lactone. Glucuronic acid forms a lactone which crystallises well. The paired acids are lævo-rotatory.

Since aniline dyes have almost entirely displaced euxanthic acid from the market the latter has become very scarce. A convenient source of glucuronic acid has been found in the menthol compound obtained in the urine of rabbits after administration of menthol. The urine is extracted with ether and ammonia added, when the ammonium salt separates (Neuberg).

According to Neuberg glucuronic acid or an isomeride is produced in small quantity when glucose is oxidised by nitric acid for the preparation of saccharic acid.

An interesting derivative of glucuronic acid is produced according to Sieburg on the administration of nitroso-phenylhydroxylamine to a dog. The new crystalline compound is decomposed by emulsin into its two components and is considered to be the lactam of *p*-aminophenolglucuronic acid.

The administration of chloralose leads to the excretion of a paired chloralose glucuronic acid from which chloralose and glucuronic acid were the only products obtained on hydrolysis (Tiffeneau).

Galacturonic Acid.

Saurez has isolated an isomeric form of glucuronic acid from lemon pulp. It gives many of the reactions of glucuronic acid but does not form a *p*-bromophenylhydrazone nor yield glucuronic anhydride; on oxidation it gives mucic acid.

A similar galacturonic acid has been discovered by Ehrlich in the pectin substances from the sugar beet. On heating pectic acid with 1 per cent. of oxalic acid, galactose galacturonic acid is obtained. Treatment of pectic acid with alkali leads to the formation of an amorphous white powder $(\alpha)_D = +270°$, $C_{24}H_{34}O_{25}$, which Ehrlich regards as a tetragalacturonic acid, viz. $4C_6H_{10}O_7 - 3H_2O$.

This gives all the reactions of the pentoses and of glucuronic acid except that on oxidation it forms mucic acid; *d*-galacturonic acid is a feebly dextro-rotatory syrup, reducing Fehling's solution in the cold,

giving furfural with hydrochloric acid and readily undergoing oxidation to mucic acid.

Pectin substances obtained from a large number of plants and vegetables have all been shown to be derivatives of this acid, and it must be regarded as playing an important part in the structure of plant material. The presence of galactose in this oxidised form in plants is of the greatest interest. Glucuronic acid is said to have been found in the sugar beet, but in view of the above it was probably mistaken for galacturonic acid.

Synthesis and Degradation.

The methods devised in the laboratory for the formation of carbohydrates containing a greater or lesser number of carbon atoms than six in the chain are of interest.

The aldoses combine directly with hydrogen cyanide forming nitriles; these, when hydrolysed, give rise to acids containing one carbon atom more than the original carbohydrate.

$$C_5H_{11}O_5 . CHO + HCN = C_5H_{11}O_5 . CH(OH) . CN \rightarrow$$
$$C_5H_{11}O_5 . CH(OH) . CO_2H \rightarrow C_5H_{11}O_5 . CH(OH) . CHO$$

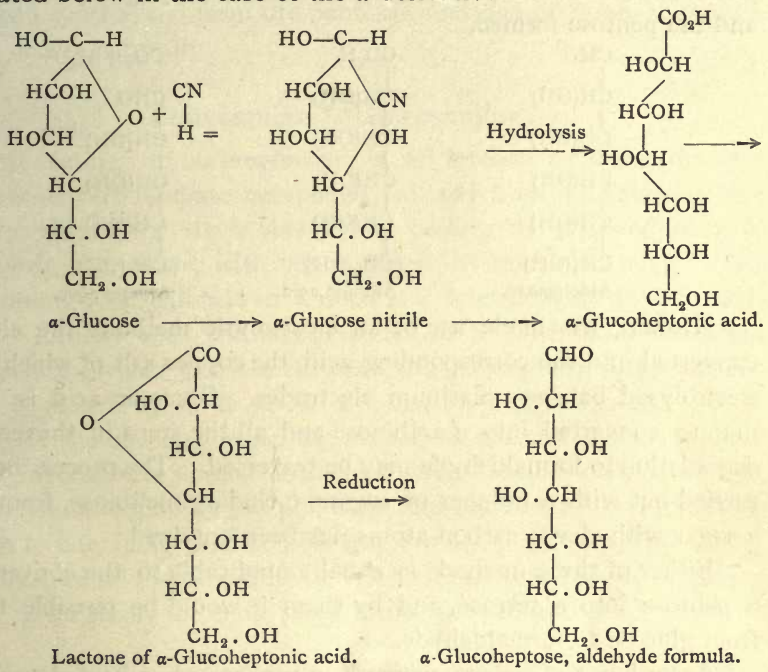
The lactones of these acids, when reduced with sodium amalgam, yield the corresponding aldoses with one carbon atom more than the original carbohydrate.

In this manner glucose can be obtained from arabinose, glucoheptose from glucose. The process has been continued by Fischer as far as the aldononoses in the case of glucose and mannose; Philippe has prepared glucodecose. It would be possible by such a method to advance step by step from formaldehyde to the highest sugars, but the operation would demand the expenditure of very large quantities of material.

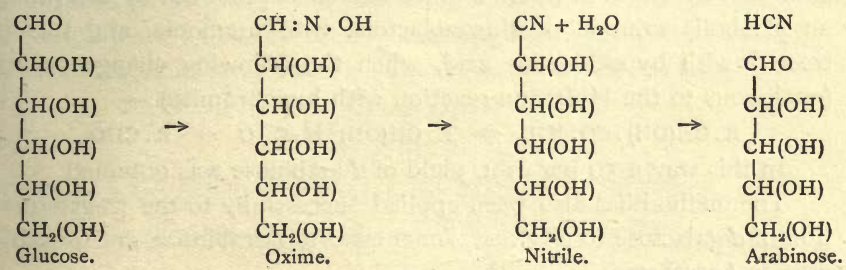
The cyanohydrin synthesis, however, is not in reality so simple as just pictured, inasmuch as usually two stereoisomeric nitriles are formed simultaneously. Arabinose gives both glucose and mannose; glucose yields two glucoheptoses. On the basis of the aldehydic formula for glucose a new asymmetric carbon atom is created in the nitrile, and, according to the ordinary rules, two forms will be produced unless the synthesis is asymmetric in character. Mannose and fructose afford the only instances at present recorded in which only one nitrile is formed.

An alternative view of the synthesis, based on the closed-ring formula, considers the two nitriles as formed simultaneously from α- and β-glucoses by a process involving first the rupture of the butylene-

oxide ring, and secondly the addition of hydrogen cyanide. The presence of α- and β-glucose in unequal proportions and the probable difference in the rate of formation of the addition product in the two cases will explain the formation of the isomeric nitriles in unequal proportions. Arabinose gives rise to a preponderance of lævo-rotatory mannonic acid, whereas from xylose and lyxose the predominating acids are lævo-rotatory. The various stages of the operation are formulated below in the case of the α-derivative—



The degradation of a sugar, i.e. the conversion into one with fewer carbon atoms, has been studied by four experimental methods. In that of Wohl the oxime of glucose is heated with concentrated sodium hydroxide and converted into the nitrile of gluconic acid, from which, on further heating, hydrogen cyanide is eliminated and a pentose—*d*-arabinose—formed. The following scheme shows the changes:—



In practice it is preferable to heat the oxime with acetic anhydride and a grain of zinc chloride: a vigorous reaction ensues, and the pent-acetate of gluconic acid nitrile is formed from which hydrogen cyanide is eliminated by treatment with ammoniacal silver oxide.

The alternative method due to Ruff makes use of Fenton's mode of oxidation with hydrogen peroxide and ferrous salts. The aldose is first converted into aldonic acid, the calcium salt of which is subjected to oxidation, with the result that the carboxyl group is eliminated and the pentose formed.

CHO		CO_2H		$CO_2 + H_2O$
|		|		|
CH(OH)		CH(OH)		CHO
|		|		|
CH(OH)	→	CH(OH)	→	CH(OH)
|		|		|
CH(OH)		CH(OH)		CH(OH)
|		|		|
CH(OH)		CH(OH)		CH(OH)
|		|		|
$CH_2(OH)$		$CH_2(OH)$		$CH_2(OH)$
Aldohexose.		Aldonic acid.		Aldopentose.

Neuberg has made use of an electrolytic method: the aldose is converted into the corresponding acid, the copper salt of which is then electrolysed between platinum electrodes. Gluconic acid is in this manner converted into *d*-arabinose and all the steps in the complete degradation to formaldehyde may be traversed. The process has been carried out with a number of sugars, including melibiose, from which a sugar with eleven carbon atoms has been obtained.

Either of these methods is equally applicable to the conversion of a pentose into a tetrose, and by them it would be possible to pass from glucose to formaldehyde.

According to Guebert, mercuric gluconate when heated undergoes intramolecular oxidation, forming *d*-arabinose in satisfactory quantity. Tollens and Böddener find, however, that this method is not applicable to the degradation of arabinose.

Weerman has contributed a new and apparently very useful method of degradation of the monosaccharides. Taking glucose-arabinose as a case in point, *d*-gluconamide is produced by saturating an alcoholic solution of gluconolactone with ammonia, and this is treated with hypochlorous acid, when the following change occurs (analogous to the Hofmann reaction with hypobromite):—

$$R.CH(OH).CO.NH_2 \rightarrow R.CH(OH).N:C:O \rightarrow R.CHO$$

In this way a 50 per cent. yield of *d*-arabinose was obtained.

The method has also been applied successfully to the transformation of *d*-galactose to *d*-lyxose, *l*-mannose to *l*-arabinose, and *l*-arabinose to *l*-erythrose.

The Aminohexoses.

Four isomeric aminoglucoses are known, viz. :—

glucosamine = 2-aminoglucose
glucosimine = 1-aminoglucose
isoglucosamine = 1-aminofructose
ε-glucosamine = 6-aminoglucose

In addition, the corresponding 2-amino derivatives of other hexoses have been synthesised from the pentoses, and one of these, chondrosamine, occurs naturally.

Chitosamine (*d*-Glucosamine).

Glucosamine, or aminoglucose, is of interest as being the first well-defined carbohydrate compound isolated from an animal tissue (Ledderhose, 1878). It is obtained by boiling the shells of lobsters, particularly the claws, with concentrated hydrochloric acid. The glucosamine hydrochloride so formed is a colourless crystalline compound. Lobster shell consists of carbonate of lime and a substance termed chitin, which yields acetic acid and glucosamine on hydrolysis. Chitin is stated by Offer to be a monacetyl diglucosamine; more recently Irvine has established the identity of the chitins derived from various invertebrate animal structures. He considers chitin to contain acetylamino-glucose and amino-glucose residues in the proportion of three to one, in agreement with the formula $(C_{30}H_{50}O_{19}N_4)_n$.

Glucosamine was obtained by Winterstein from fungus cellulose; indeed chitin seems to be the most important cell-wall material of the fungi.

The glucosamine in *Boletus edulis* is considered by Ross to pre-exist as a glucoprotein and not as a glucoside. Two glucosamine residues are said to be present in lycoperdin isolated from the fungus *Lycoperdum gemmatum*. Glucosamine is a constituent of the mucins and mucoids.

Irvine considers that it is still an open question whether glucosamine is derived from glucose or mannose, though he inclines to glucose. Levene's work on the synthetic amino-sugars makes the mannose formula much more probable. As aminoglucose it has the formula :—

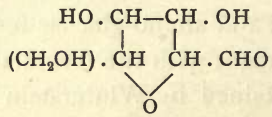
$$\begin{array}{l} \quad H\ \ H\ \ OH\ H \\ CH_2OH.\ C\ .\ C\ .\ C\ .\ C\ .\ CHO \\ \quad OH\ OH\ H\ \ NH_2 \end{array}$$

which is more properly written in the pentaphane ring form,

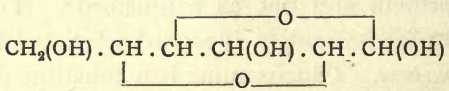
$$CH_2(OH).CH(OH).CH.CH(OH).CH(NH_2).CH(OH)$$
$$\qquad\qquad\qquad

Glucosamine is prepared from the hydrochloride by decomposing it with diethylamine (Breuer) or sodium methoxide (Lobry de Bruyn). It derives special interest from the fact that it may be regarded as a link between the carbohydrates and *a*-hydroxyamino acids. The synthesis of glucosamine, by Fischer and Leuchs, which at the same time established its constitution, thus becomes of enhanced importance. By the combination of *d*-arabinose and ammonium cyanide, or of *d*-arabinoseimine with hydrogen cyanide, *d*-glucosamic acid, $CH_2(OH).[CH.OH]_3.CH(NH_2).CO_2H$, was obtained and its lactone reduced to glucosamine. Glucosamine forms a penta-acetyl derivative and also an oxime, semi-carbazone and phenyl hydrazone, but it cannot be converted into glucose, though it gives glucose phenyl osazone when heated with phenyl hydrazine. Nitrous acid converts it into a compound ($C_6H_{10}O_5$) formerly regarded as a sugar and termed chitose; this forms chitonic acid when oxidised. Glucosamine is often regarded as a derivative of chitose, and termed chitosamine. It is perhaps desirable to retain this name until its identity as glucosamine or mannoseamine has been established.

Chitose was claimed by Fischer and Andreae to be a hydrated furfurane derivative rather than a true sugar, formed by simultaneous elimination of the amino group and anhydride formation. It has the formula—



Irvine and Hynd formulate chitose (anhydro-glucose) with the aldehydic radicle present, as in the hexoses, in the butylene oxide form—



whereas Levene and La Forge consider it to be *a*-δ-anhydromannose. Accordingly, chitonic acid is *a*-δ-anhydromannonic acid whilst the isomeric chitaric acid formed by the action of nitrous acid on glucosamic acid is considered to be *a*-δ-anhydrogluconic acid.

Irvine has prepared an amino methyl glucoside from glucosamine and converted it into glucose, thus establishing the relationship between glucose and glucosamine. The conversion takes place through the following reactions: *d*-glucosamine hydrochloride→bromotriacetyl glucosamine hydrobromide→triacetyl amino methyl glucoside hydrobromide→amino methyl glucoside hydrochloride.

This last compound, like other derivatives of glucosamine, reacts abnormally with nitrous acid and does not yield methyl glucoside, On methylation by the silver oxide method dimethyl amino methyl glucoside is obtained, from which the substituted amino group is expelled by heating with barium hydroxide. The product is further methylated and converted into tetramethyl methyl glucoside, from which *d*-glucose results on removal of the methyl groups.

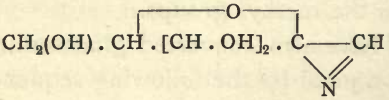
Irvine and Hynd have also converted glucosamine into *d*-mannose in almost quantitative yield by the following sequence of reactions :—

d-Glucosamine hydrochloride→methylglucosamine hydrochloride→ benzylidene methylglucosamine hydrochloride→a benzylidene hexose→ *d*-mannose. A Walden inversion might take place during the course of these changes, and probably in the formation of the benzylidene hexose, which is effected by the action of sodium nitrite upon the preceding amino-compound.

Glucoseimine, first obtained by Lobry de Bruyn, is prepared by the action of ammonia on glucose dissolved in methyl alcohol. It has been held at various times to be an iminoglucose or a nitrogen ring compound, but the bulk of the evidence available points to it being a 1-amino-glucose—

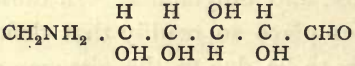
$$CH_2(OH).CH(OH).CH.(CH.OH)_2.CH.NH_2,$$
$$\quad\quad\quad

A second product—$C_6H_9O_4N$—is also formed during this reaction. It is converted into glucosephenylosazone without difficulty and is produced also by the action of ammonia in methylalcoholic solution on glucosone. It is stable towards acids but the nitrogen is easily expelled as ammonia by alkalis. Irvine terms it fructoseazine and assigns to it the formula—

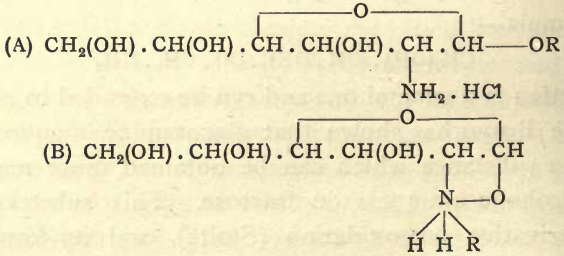


deriving it from the unknown fructoseimine by the elimination of water.

An isomeride of glucosamine has been obtained by Fischer by the following series of operations: β-pentacetyl glucose, when treated with anhydrous liquid hydrogen bromide, forms dibromo-triacetyl glucose which reacts with methyl alcohol to give triacetyl β-methyl glucoside bromohydrin. This is converted by ammonia at the ordinary temperature into amino β-methyl glucoside from which the amino sugar is obtained on hydrolysis. The new compound reduces Fehling's solution but differs from glucosamine in a number of ways, the osazone which it yields with phenyl-hydrazine being different from phenyl-glucosazone; it is also less stable to acids. Judging from the production of an anhydro glucose from dibromo-triacetyl glucose (p. 28) the amino group in the new isomeride is attached to the carbon atom in the terminal position thus:—



Irvine and Hynd have obtained synthetic aminoglucosides by condensing bromo-triacetylglucosamine hydrobromide with an hydroxy compound in presence of a base; glucosides of two types are produced:—

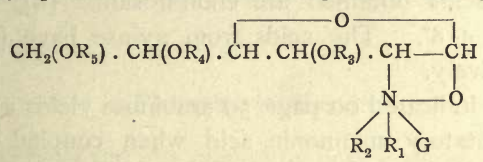


When the group condensed with the glucosamine residue consists of a short open chain the product possesses properties similar to those of α-aminomethylglucoside and thus belongs to type B, which includes

a modified betaine ring in the molecule. Derivatives which contain a benzene nucleus in the glucosidic position behave as true glucosides and behave as type A.

Compounds of type B are remarkably stable towards the hydrolytic action of acids, they are not hydrolysed by emulsin. The normal aminoglucosides are easily hydrolysed by acids and both aminosalicin and aminohelicin are hydrolysed by emulsin.

The wide distribution of betaines in nature makes the synthetic aminoglucosides of type B of special interest, the more so as they may be closely related to glucoproteins. Irvine regards these last as glucosamine derivatives in which aminoacyl residues, i.e. short polypeptide chains (R_1, R_2, R_3, etc.), occupy the amino position and possibly all the hydroxyl positions with the exception of the glucosidic group.



Much of the behaviour of the mucins is in agreement with this hypothesis.

Irvine's observations receive support from the fact that when glucosamic acid, the oxidation product of glucosamine, is methylated by means of methylsulphate and barium hydroxide the molecule undergoes fission, the products, according to Pringsheim, being betaine and an uncrystallisable solid, consisting probably of a methylated tetrose. This observation is of interest in view of the possible connection of such an action with the occurrence of betaine and of proteins in the sugar beet.

Chondrosamine.

Cartilage (nasal septa) contains a substance, chondroitin sulphuric acid; this is a tetrasaccharide consisting of two glucuronic acids and two chondrosamine units, in which the amino groups are acetylated. Chondrosamine has been the subject of an extensive investigation by Levene and La Forge. It was at first described as glucosamine but proved to be an isomeride of this and was considered to be *l*-allosamine or *l*-altrosamine owing to the supposed similarity between the osazones of chondrosamine and these hexoses. The osazone was subsequently identified as galactosazone, and chondrosamine shown to be identical with the synthetic lyxohexosamine, both amino sugars yielding anhydromucic acid on oxidation and forming identical derivatives. It is thus either 2-amino-galactose or 2-amino-talose, probably the latter.

$$CH_2(OH) \cdot \begin{matrix} H & OH & OH & NH_2 \\ C & . & C & . & C & . & C & . & CHO \\ OH & H & H & H \end{matrix}$$

The aminohexoses are synthesised from the pentoses by the following series of operations. By means of methylalcoholic ammonia the imine (1-aminopentose) is formed which is left with hydrocyanic acid and the product hydrolysed with hydrochloric acid at 0° C. A mixture of the two epimeric hexosamic acids is obtained in this manner and oxidation with nitric acid converts these into the corresponding tetrahydroxyadipic acids. The epimeric acids can be converted into one another by heating with pyridine in the usual manner.

The two *d*-arabinohexosamic acids correspond to chitosamic acid (from glucosamine) $[a]_D$ − 15° and the epimeride $[a]_D$ − 10°. From *d*-lyxose the acids obtained are chondrosamic $[a]_D$ − 17° and the epimeride $[a]_D$ + 8°. The acids from xylose have $[a]_D$ + 14° and − 11° respectively.

As already indicated on page 59 arabinose yields a preponderance of the lævo-rotatory mannonic acid when coupled with hydrogen cyanide, whereas lyxose and xylose yield mainly dextro-rotatory hexonic acids. Similarly, 1-aminoarabinose yields a larger proportion of a lævo-rotatory hexosamic acid (identical with glucosamic acid), whereas dextro-rotatory hexosamic acids preponderate from aminolyxose and aminoxylose. Levene considers the analogy to justify the regarding of the acid from arabinose as mannosamic acid.

The configuration of these synthetic *a*-hexosamic acids is also indicated by optical considerations. In each pair of hexonic acids the member which has the same configuration of the *a*-carbon atoms as *d*-gluconic acid possesses either a higher dextro-rotation or a lower lævo-rotation than the epimeride. By heating the acid with pyridine an increase in rotation occurred with each of the acids excepting lyxohexosamic acid. Hence chitosamic acid (from glucosamine) has the configuration of mannosamic acid, xylohexosamic acid that of idosamic acid, chondrosamic acid that of talosamic acid, and *d*-lyxohexosamic acid that of galactosamic acid. The value of the *a*-carbon atom in each pair of epimeric acids is found by experiment to be 12·5°.

Levene and La Forge consider chondroitin to be a tetrasaccharide consisting of two glucuronic acids and two chondrosamine units. The amino groups are acetylated and the primary alcohol groups esterified with sulphuric acid. On hydrolysis the sulphuric and acetic acids are split off and the molecule ruptured between the two glucuronic acid molecules forming chondrosin. Levene has obtained mucoitin sulphuric acid from a number of mucins and mucoids. This differs

from chondroitin since on hydrolysis it yields a disaccharide composed of glucuronic acid and glucosamine which is termed mucosin :—

$$C_{12}H_{21}O_{11}N$$

Hudson has isolated the α- and β-pentacetates of glucosamine and chondrosamine and contrasted their molecular rotations with those of the glucose pentacetates. The end asymmetric carbon has the same configuration in all though it is reversed in chondrosamine, which is a derivative of an *l*-sugar, and, as the table on p. 41 shows, the values of 2A for the amino-sugars agree closely. The agreement is not quite so good with the glucose pentacetates which may indicate that the nature of the groups on the chain have in this case a definite though small influence upon the rotation of the end asymmetrical carbon atom.

Phosphoric Esters.

The discovery of the rôle played by hexose phosphate in fermentation lends considerable interest to the phosphoric esters of carbohydrates.

The hexose phosphate $C_6H_{10}O_4(PO_4H_2)_2$ from glucose, mannose or fructose (see p. 116) is not precipitated by ammoniacal magnesium citrate mixture, but the lead salt is precipitated by lead acetate. It can be purified by decomposition by hydrogen sulphide and reprecipitation. With phenyl hydrazine an osazone is formed, one molecule of phosphoric acid being eliminated, which has the composition—

$$(H_2PO_4)C_6H_6(OH)_3(NHPh)_2$$

The sodium, phenyl hydrazine and aniline salts have been characterised.

Hexose phosphoric acid contains an active carbonyl group and two phosphoric acid groups, one of the latter being probably attached to the carbon atom adjacent to the carbonyl group since it is split off in the formation of the osazone.

Neuberg has described phosphoric esters of glucose and sucrose prepared by the action of phosphorus oxychloride on the carbohydrates in presence of calcium carbonate or hydroxide. These have the composition $C_6H_{11}O_5 . O . PO_3Ca$ and $C_{12}H_{21}O_{10} . O . PO_3Ca$. Neither of them is fermented by yeast. On the other hand, the corresponding calcium fructose phosphate obtained by partly hydrolysing sucrose phosphate with dilute hydrochloric acid is stated to be readily fermented by yeast. It reduces Fehling's solution.

Phosphoric acid esters of the carbohydrates play an important part in the structure of the nucleic acids. Thus inosinic acid when

hydrolysed in acid solution yields a purine base and *d*-ribose phosphoric acid. The position where pentose and acid are attached is not known. Yeast nucleic acid contains this grouping four times, whilst in thymus nucleic acid the sugar is a hexose.

Tannins.

The tannins have long been regarded as glucosides, Strecker in 1852 being the first to show that they contained glucose. His formula, $C_{27}H_{22}O_{11}$, for tannin corresponded with three molecules of gallic acid to one of glucose. Other observers have disputed the presence of glucose in tannin, which often figures simply as digallic acid in the older textbooks. Statements as to the amount of glucose obtained from tannin on hydrolysis vary very widely: this is due to the great difficulty experienced both in purifying the tannin and in separating the glucose formed. Fischer and Freudenberg (1912) showed that carefully purified tannin yields somewhat more than 8 per cent. of glucose on hydrolysis. This proportion is too small for tannin to be a glucoside of the ordinary type, but it is suggested by Fischer and Freudenberg that it is an acyl derivative of glucose analogous to pentacetylglucose or pentabenzoylglucose. A pentadigalloylglucose,

$$CH_2(OX).CH(OX).CH.CH(OX).CH(OX).CH(OX)$$
$$\text{(bridge O)}$$

where

$$X = -CO.C_6H_2(OH)_2.O.CO.C_6H_2(OH)_3$$

should contain 10·6 per cent. of glucose. It has the high molecular weight 1700. This formula is in agreement with what is known as to the composition, optical activity, small acidity and the behaviour of tannin on hydrolysis.

Proof of the correctness of this hypothesis is afforded by the synthesis by Fischer and Freudenberg of acyl derivatives of glucose closely analogous to natural tannin. On shaking glucose with a chloroform solution of trimethylcarbonato galloylchloride in presence of quinoline an acyl derivative is formed from which, on cautious hydrolysis with alkali, the methylcarbonato groups can be removed so that pentagalloylglucose is formed. The synthetic compound has all the properties of the tannins. Other phenolcarboxylic acids may be used for the condensation, and methyl-glucoside or glycerol may be substituted for glucose. The way is thus opened for the synthesis of a variety of products of high molecular weight, amounting in the extreme case of derivatives of the disaccharides to several thousands. It is quite possible that such compounds may be present in animals.

Fischer and Bergmann have succeeded in synthesising penta-(*m*- and *p*-digalloyl)-β-glucoses, the former of which is remarkably similar to Chinese tannin, the only point of difference noted being the specific rotation, which is of minor importance in colloid substances of such complexity.

Glucose in combination with gallic acid has been shown by Feist to be associated naturally with tannin. The natural compound differs from the β-glucosidogallic acid, $C_6H_{11}O_5 . O . C_6H_2(OH)_2 . CO_2H$, prepared by Fischer and Strauss in which the coupling of the hexose and the aromatic residue involves a phenolic group, as is confirmed by its conversion into glucosyringic acid. It is suggested that in the natural compound the carboxyl group is involved—

$$C_6H_{11}O_5 . O . CO . C_6H_2(OH)_3,$$

and this has been confirmed by the preparation of a 1-galloylglucose having the above structure by Fischer and Bergmann which is in all respects identical with the natural glucogallin isolated by Gilson from Chinese rhubarb, though it is said to be quite distinct from Feist's glucogallic acid.

CHAPTER III.

THE HEXOSES, PENTOSES AND CARBOHYDRATE ALCOHOLS.

THE general properties of the monosaccharides have been fully dealt with in the foregoing and exemplified in the case of glucose. In dealing with the remaining hexoses it is only necessary to recapitulate briefly their more important properties and any salient points of difference from glucose. Most of the synthetic sugars are not referred to in detail as they are not of biochemical interest.

Glucose and fructose are the only two of the monosaccharides which occur naturally as such. The others are found in nature as polymerides, as glucosides, or in the form of alcohols, and are prepared by hydrolysis or oxidation.

Fructose and sorbose are types of the ketohexoses, a group which has been much less investigated than the aldohexoses. Both fructose and sorbose have the ketonic oxygen attached to the α-carbon atom, but a number of other isomerides are possible in which the keto group is situated elsewhere in the molecule. The ketohexoses do not yield acids containing the same number of carbon atoms on oxidation, but the molecule breaks into two at the ketonic group.

TABLE X.—THE MONOSACCHARIDES.

Diose.	*Trioses.*	*Tetroses.*
Glycollic aldehyde.	*d*- and *l*-Glycerose.	*d*- and *l*-Erythrose.
	Dihydroxyacetone.	*d*- and *l*-Threose.
	Methylglycerose.	Erythrulose.

Pentoses.	*Methylpentoses.*
d- and *l*-Arabinose.	*l*-Rhamnose.
d- and *l*-Xylose.	*d*- and *l*-Isorhamnose.
d- and *l*-Ribose.	Fucose, Rhodeose.
d- and *l*-Lyxose.	Epirhodeose.

Aldohexoses.	*Ketohexoses.*
d- and *l*-Glucose.	*d*- and *l*-Fructose.
d- and *l*-Mannose.	*d*- and *l*-Sorbose.
d- and *l*-Gulose.	Tagatose.
d- and *l*-Idose.	
d- and *l*-Galactose.	
d- and *l*-Talose.	
d-Altrose.	
d-Allose.	

TABLE X.—THE MONOSACCHARIDES—*continued.*

Heptoses.	*Octoses.*	*Nonoses.*	*Decose.*
Mannoheptose.	Manno-octose.	Mannononose.	Glucodecose.
Glucoheptose.	Gluco-octose.	Gluconononose.	
Galactoheptose.	Galacto-octose.		
Mannoketoheptose.			
Perseulose.			
Sedoheptose.			

Mannose.

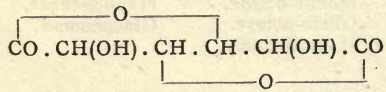
d-Mannose is widely distributed in nature in the form of anhydride-like condensation products termed mannosans which are converted into mannose when hydrolysed by acids; it does not occur in more simple form. A convenient source for its preparation is the vegetable ivory nut. This is the endosperm of the seed of the tagua palm *Phytelephas macrocarpa.* A method of obtaining as much as 4 per cent. of the weight of the vegetable ivory in the form of pure crystalline mannose has been worked out in detail by Hudson. The meal is hydrolysed by 75 per cent. sulphuric acid, the acid removed by means of calcium hydroxide, and after purification the colourless solution is evaporated to a thick syrup and this mixed with an equal volume of glacial acetic acid. This method makes a great advance on the original practice of Fischer and Hirschberger of isolating the phenyl-hydrazone and decomposing this to the sugar, and makes the sugar available for the further study of its properties.

Mannose forms rhombic crystals and has initially a sweet taste followed immediately by a distinctly bitter one. This bitterness is quite characteristic and of interest, in view of the pure sweetness of the closely related isomerides glucose and fructose, the latter being the sweetest sugar known.

Crystalline mannose is the β form having $[\alpha]_D - 17°$. The rotation of the α form is calculated by Hudson to be $+ 34°$ and that of the equilibrated mixture is $+ 14{\cdot}6°$.

Mannose is the true aldehyde of mannitol, and may be obtained from it by oxidation, or converted into it by reduction. It is of interest that it was first prepared by Fischer and Hirschberger in this manner, and only subsequently identified as a natural product. It is very similar to *d*-glucose in its general properties, exhibits mutarotation, and forms the same phenyl osazone as glucose and fructose. Mannose is altogether remarkable in forming a sparingly soluble phenyl hydrazone which enables it to be very easily identified. This hydrazone is precipitated within a few minutes when phenyl hydrazine is added to a solution of mannose.

Nitric acid oxidises mannose to *d*-mannosaccharic acid, which readily forms a double lactone :—



Mannose forms an additive compound with hydrogen cyanide, which, on hydrolysis, yields mannoheptonic acid. Apparently one only of the two possible isomerides is formed. The mannoheptose obtained from this is very similar to mannose, and forms a sparingly soluble phenyl hydrazone. On reduction it yields the alcohol $C_7H_{16}O_7$ identical with the natural perseitol.

Galactose.

d-Galactose occurs as a constituent of milk sugar and raffinose, also in many gums and seaweeds as the polymeric form galactan; its presence in the form of a galactoside is rare, being confined to the saponins, xanthorhamnin, and a few other natural glucosides. Lippmann records the appearance of galactose as a crystalline efflorescence resembling hoar frost on ivy berries following a sharp frost, the first after a late dry autumn. Both isomeric forms of galactose occur naturally: Winterstein found *dl*-galactose in Chagnal gum, Tollens obtained it from Japanese Nori.

The galactans are widely distributed in the form of gums, mucilages, pectins. Galactose is usually associated in them with arabinose or xylose. The pectins, which are of importance in the jam industry, are hydrolysed by acids to galactose and arabinose and by an enzyme pectase into pectic acids. It resembles glucose in properties; characteristic is the formation of mucic acid on oxidation with nitric acid, and this may be used for its identification. On reduction with sodium amalgam the corresponding alcohol, dulcitol, is formed; this is found naturally. By the action of alkalis it is transformed into *d*-talose and *d*-tagatose. It is fermented by some yeasts, but not by all those which ferment glucose ; a fact which has been taken as indicating that a special galacto-zymase is required for the fermentation.

Ordinary galactose is the α form $[\alpha]_D + 144°$; it crystallises in anhydrous leaflets or from water as the hydrate in large prisms. The β form has $+ 52°$ and the equilibrium mixture $+ 80°$. α-Methylgalactoside is not hydrolysed by enzymes; β-methylgalactoside is attacked, like milk sugar, by the lactase of kephir, by the lactase present in some yeasts, and by a lactase present in an aqueous extract of almonds (see Chapter V.).

The γ form of methylgalactoside has recently been isolated and corresponds in general behaviour to the ethylene oxide form of methylglucoside; it is of interest that its tetramethyl derivative, after removal of the glucosidic methyl group by gentle hydrolysis, undergoes auto-condensation to an octamethyldigalactose.

Under abnormal conditions galactose is formed in the sugar beet, and appears in combination with sucrose as the trisaccharide, raffinose. The quantity of raffinose is increased abnormally by disturbances of growth, such as those occasioned by sudden frost. Under these conditions the galactans are supposed to undergo hydrolysis and form galactose. Apparently the plant, when confronted with galactose, utilises it first to form a disaccharide, melibiose, composed of glucose and galactose, and then makes use of the glucose half in this disaccharide, according to its fixed habit, by combining it with fructose, with the result that a compound carbohydrate containing all three simple hexoses is formed.

Galactose is the sugar of the brain whence it was isolated and described under the name cerebrose by Thudichum. It is a constituent of the cerebrosides known as phrenosin and kerasin.

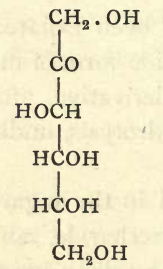
These compounds on hydrolysis furnish galactose, a base sphingosine and a fatty acid; that in phrenosin being phrenosinic acid, $C_{25}H_{50}O_3$, and that in kerasin being lignoceric acid, $C_{24}H_{48}O_2$. They are both optically active and have the property of forming liquid crystals. (For further details see the monograph by Maclean in this series.)

Fructose.

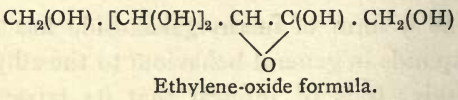
d-Fructose or lævulose, discovered by Dubrunfaut in 1847, occurs together with glucose in the juices of fruits, etc., the mixture being often termed fruit sugar or invert sugar. It is present in chicory and especially the Jerusalem artichoke. Combined with glucose it occurs as cane sugar, raffinose, etc. It is a constituent of alliin, the glucoside of garlic and of some saponins. The polysaccharide inulin yields fructose alone when hydrolysed.

To prepare it from invert sugar or hydrolysed inulin it is best to form the crystalline calcium lævulosate and decompose this with carbon dioxide.

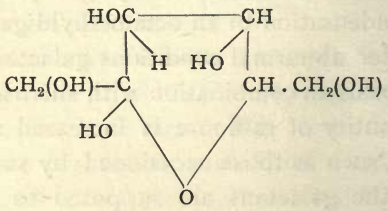
Fructose is a ketohexose having the following alternative constitution :—



Ketonic formula.



Ethylene-oxide formula.



Butylene-oxide formula.

Fructose crystallises less easily than glucose, and its derivatives are also difficult to crystallise. It is much sweeter than glucose. It exhibits mutarotation, and, like glucose, exists in solution presumably as an equilibrated mixture of several stereoisomeric forms. It is remarkable for the very large change produced in the specific rotatory power by changes of temperature. The rotatory power becomes less negative as the temperature is increased, and at 87·3° C. it is equal and opposite to that of glucose.

The recent work of Irvine and his school has afforded evidence that fructose is much more prone than is glucose to react in the ethylene oxide form. Pure solid fructose which has been dissolved in water is quite stable to permanganate and represents the butylene-oxide form, but if the solution is made acid, kept for an hour and neutralised, permanganate is decolorised within a few minutes, showing that the ethylene-oxide form of fructose has been formed. Ethylene-oxide itself in solution behaves similarly towards permanganate. Fructose thus reacts either as—

(1) A compound containing the butylene-oxide ring and existing in α and β modifications. Ordinary fructose, the β modification, forms rhombic crystals, $[\alpha]_D$ − 133·5° falling to −92° in solution. The calculated rotation of the α form is − 21°.

This type of fructose is not attacked by permanganate, does not combine readily with acetone and forms stable fructosides and acetyl derivatives, e.g. a pentacetate and tetracetyl β-methyl fructoside, also a crystalline tetramethyl-fructose $[\alpha]_D$ − 125°.

(2) A more active compound probably containing an ethylene-oxide ring and likewise capable of existing in interconvertible α and β modifications having lower specific rotations. The derivatives of this form are highly reactive, combine readily with acetone, and reduce permanganate. The fructosides are very easily hydrolysed

by acids and resemble sucrose, which is perhaps a derivative of the ethylene-oxide fructose. The tetramethyl derivative is a liquid having $[\alpha]_D + 29{\cdot}3°$.

When submitted to the action of ultra-violet rays, solutions of fructose are transformed into carbon monoxide and dioxide, formaldehyde, methyl alcohol, and aldehydic and acidic substances; this is said to be the first degradation of fructose which has been effected by other than purely chemical or biochemical agency.

Fructose shows a number of characteristic reactions. Hydrogen bromide interacts with fructose in ethereal solution to form bromomethylfurfuraldehyde $\begin{matrix} CH : C(CH_2Br) \\ | \\ CH : C(CHO) \end{matrix}\!\!>\!O$, a substance which crystallises in golden yellow rhombic prisms; the ethereal liquid is coloured an intense purple-red (Fenton and Gostling). A β-oxy-γ-methylfurfuraldehyde is produced on heating concentrated solutions of fructose under pressure, preferably with oxalic acid.

On prolonged boiling with dilute mineral acids, lævulinic acid, $CH_3 . CO . CH_2 . CH_2 . CO_2H$, is formed together with formic acid and humus substances.

When oxidised by means of mercuric oxide fructose forms glycollic acid, $CH_2(OH) . CO_2H$, and trihydroxybutyric acid, $CH_2OH . (CH . OH)_2 . CO_2H$. It is not acted upon by bromine water of low concentration: aldoses can be distinguished from ketoses by means of this test. Mannitol and sorbitol are formed on reduction with sodium amalgam.

By the action of methyl alcohol and hydrogen chloride on fructose a syrup is obtained which probably represents a mixture of methyl fructosides, in which undoubtedly both α and β modifications of the ethylene and butylene-oxide forms are present. On methylation and hydrolysis a mixture of tetramethylfructoses is obtained, which has been separated into the syrupy, ethylene-oxide and the crystalline, butylene-oxide forms. The original syrupy mixture could not be obtained crystalline.

This syrup is partially hydrolysed by yeast extract, but, inasmuch as Pottevin has shown that it is not hydrolysed by *S. octosporus*, *Mucor mucedo* and other ferments which attack cane sugar and maltose, the hydrolysis is presumably caused by an enzyme other than invertase or maltase, neither of which should act on these fructosides (see Chapter IV.).

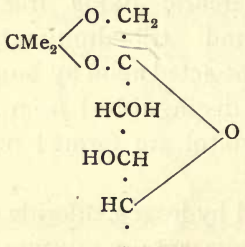
Crystalline β-methylfructoside, $[\alpha]_D - 172°$, was obtained by Hudson by methylation of tetra-acetyl fructose and subsequent hydrolysis.

It is not hydrolysed by the enzymes of yeast nor by emulsin and does not show mutarotation. It undoubtedly has the butylene-oxide structure.

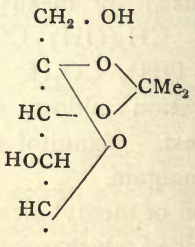
Fructose, like glucose, forms an additive compound with hydrogen cyanide which yields fructose carboxylic acid on hydrolysis ; this, when boiled with hydriodic acid, is converted into methyl butylacetic acid, $C_4H_9 . CHMe . CO_2H$. This reaction and the behaviour on oxidation establish the formula of fructose.

Fructose forms the same osazone as glucose; it also forms osazones with some disubstituted phenyl hydrazines, the primary $CH_2(OH)$ group being more easily oxidised by these than the secondary CH(OH) group in glucose. The methyl phenylosazone is characteristic of fructose.

Glucose and its isomerides combine with acetone in presence of hydrogen chloride forming mono- and diacetone derivatives of a glucosidic nature since they no longer reduce Fehling's solution. Enzymes are entirely without action on them. The acetone compounds of fructose have been investigated by Irvine who has proved the existence of two isomeric fructose monacetones—



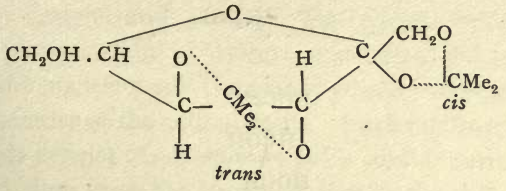
A



B

having probably the formulæ A and B, each of which will exist in *a* and *β* forms. From A a diacetone is formed, but B is not prone to further condensation : this is consistent with the view that the acetone $\rangle CMe_2$ residue displaces the hydrogen atom of two adjacent hydroxyl groups which need not, however, be on the same side of the formula as represented on a plane surface.

The expressions *cis* and *trans* are used by Irvine to distinguish between the linkage between hydroxyl groups on the same or on opposite sides of the molecule. In fructose diacetone both types are present.



It is not therefore surprising that the two acetone groups are eliminated at different rates on hydrolysis, fructose *cis* monoacetone being formed as an intermediate product. In triacetone mannitol there is evidence that the acetone groups are in order *trans*, *trans*, *cis*, and di- and monoacetone compounds are formed in turn on cautious hydrolysis. The most stable acetone residue is attached to a terminal primary alcohol group.

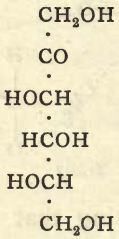
It is probable that glucose and fructose play distinct parts in metabolism. Brown and Morris have shown that glucose is mainly concerned in respiration; fructose appears to take part more particularly in the elaboration of tissue since it is far less stable than glucose.

In this connection the experiments of Lindet are of particular interest. Dealing more particularly with yeasts and moulds he adduces experimental evidence to prove that fructose is specially concerned in tissue formation, glucose being more readily used for fermentation and respiration. Yeasts and moulds, for equal weights of sugar consumed, show greater growth in fructose and they consume glucose preferentially from invert sugar.

It is stated also that fructose is sometimes found to be assimilated by diabetics when glucose is inadmissible.

Sorbose.

Sorbose was discovered by Pelouze in 1852 and was isolated from the juice of mountain ash berries which had been exposed to the air for many months. These berries contain the alcohol sorbitol, which, under the influence of an oxidising organism, shown by Emmerling to be identical with the bacterium xylinum of Adrian Brown, is oxidised to sorbose. The brilliant researches of Bertrand have given a complete explanation of the transformation, and have rendered the preparation of sorbose a relatively simple matter. Sorbose is a ketose having the formula—



It has a marked crystallising power, is not fermentable, and generally behaves as fructose; on reduction it yields sorbitol. Lobry de Bruyn has shown that under the influence of alkali sorbose is converted into *l*-gulose, *l*-idose and *l*-galactose, and so affords a connecting link between hexoses of the mannitol and dulcitol series. This result is of importance, as the direct synthesis of a hexose of the dulcitol series has not been achieved.

Fischer originally designated it as *d*-sorbose because on reduction it yields the same sorbitol as *d*-glucose. Both Rosanoff and Hudson suggest the name *l*-sorbose on account of the structural relationship to *l*-glycerose and *l*-glucose: their contention is undoubtedly correct.

a-Methyl-*l*-sorboside, $[a]_D$ – 88°, obtained by the action of methyl alcohol and hydrochloric acid on *l*-sorbose, is so named by Hudson to fit in with his contention that the *a*-isomeride of a lævo sugar is the more lævo-rotatory.

The antipode *d*-sorbose has been prepared by Lobry de Bruyn and van Ekenstein by the partial transformation of *d*-galactose under the influence of dilute alkalis. It has $[a]_D$ + 42·9°.

The Pentoses $C_5H_{10}O_5$.

Two pentoses, *l*-arabinose and *d*-xylose,[1] are widely distributed in the vegetable kingdom as polysaccharides of high molecular weight, the so-called pentosans; they also occur in complex glucosides, but are never found as the simple sugars. Xylose is found in straw, oat hulls and in most woods; arabinose in gums, being conveniently prepared from cherry gum or gum-arabic. The *d*-isomeride of arabinose can be obtained synthetically from *d*-glucose by the degradation methods indicated in the previous chapter. Recently it has been found naturally as a constituent of the glucoside barbaloin, and described under the name aloinose (Léger).

In the animal kingdom a pentose is a constituent of the nucleoproteins and nucleic acids. The nature of this pentose has been a subject of controversy; it is now regarded as *d*-ribose and identical with

[1] Hitherto known as *l*-xylose.

the carnose of Levene and Jacobs. The nucleic acids consist of a carbohydrate, phosphoric acid, two purine bases and two pyrimidine bases. In plant nucleic acids the carbohydrate is *d*-ribose and this is also the only pentose of the animal body. Such nucleotides as guanylic or inosinic acids consist of phosphoric acids and a purine base united by ribose, and they form the corresponding nucleosides consisting of ribose and purine base when submitted to neutral hydrolysis at 175° under pressure. The nucleosides are glucosides (pentosides) and are decomposed by boiling mineral acids. Similar compounds of the pyrimidine bases exist. (For further details see the monograph by Walter Jones in this series.)

The carbohydrate group obtained from thymus nucleic acid is generally regarded as lævulinic acid, which can be formed by heating hexoses with sulphuric acid. It is regarded as of secondary origin from a hexose group. Lævulinic acid is obtained uniformly from animal nucleic acids but never from plant nucleic acids. The recent work of Feulgen makes it very probable that the carbohydrate group consists of glucal, $C_6H_{10}O_4$,

As described later glucosides of carbohydrate and purine have been prepared synthetically.

A pentose appears as an abnormal product in urine in the rare disease pentosuria—according to Neuberg this is inactive *dl*-arabinose (see Garrod, "Inborn Errors of Metabolism").[1]

But little is known of the mechanism of the formation of pentoses in plants; they may be formed in the same manner as the hexoses, but independently of these, or they may be degradation products of the hexoses (cp. p. 46). Xylose and arabinose serve as nutrient to yeast and bacteria, but higher plants have no power of utilising them.

The pentosans are resistant towards alkali and require prolonged heating with mineral acids to effect hydrolysis. They are comparable with starch and cellulose and contain as a rule both C_5 and C_6 carbohydrates. No enzymes are known as yet which hydrolyse them; inasmuch as they are present essentially as skeletal, and not as food products in the plants, it is to be expected that they will be outside the range of the ordinary plant enzymes.

Their origin and function in plants has been studied recently by Ravenna, who concludes that the simple sugars more than the complex carbohydrates exert a preponderating influence on their formation. They can act as a reserve material when the plant has exhausted the more readily utilisable food-stuffs. In leaves the pentosans increase

[1] But Zerner regards it as *l*-lyxose or the corresponding xylo-ketose.

in amount during the day, decrease during the night. They increase when the leaves are supplied with glucose, diminish when the action of the chlorophyll is prevented and carbohydrate nutriment is absent.

The eight possible aldopentoses are given in the following table, together with their configuration formulæ. The table also contains the configuration formulæ of the remaining lower members of the group of monosaccharides, viz. 4 tetroses and 2 trioses :—

TABLE XI.

ALDOPENTOSES.

CHO HO|H HO|H HO|H CH_2OH *l*-Ribose.	CHO H|OH H|OH H|OH CH_2OH *d*-Ribose.	CHO H |OH HO| H H |OH CH_2OH *d*-Xylose.	CHO HO| H H |OH HO| H CH_2OH *l*-Xylose.
CHO H |OH HO| H HO| H CH_2OH *l*-Arabinose.	CHO H |OH H |OH HO| H CH_2OH *l*-Lyxose.	CHO HO| H H |OH H |OH CH_2OH *d*-Arabinose.	CHO HO| H HO| H H |OH CH_2OH d-Lyxose.

ALDOTETROSES.

CHO HO|H HO|H CH_2OH *l*-Erythrose.	CHO H|OH H|OH CH_2OH *d*-Erythrose.	CHO H |OH HO| H CH_2OH *l*-Threose Unknown.	CHO HO| H H |OH CH_2OH *d*-Threose.

ALDOTRIOSES.

CHO H|OH CH_2OH *d*-Glycerose.	CHO HO|H CH_2OH *l*-Glycerose.

The optically active glyceroses (glyceraldehydes) have been synthesised by Wohl. The dextro-rotatory form has a_D + 13° to 14°. By the hydrogen cyanide synthesis it is converted into a trihydroxybutyric acid which yields *l*-tartaric acid on oxidation.

CHO H|OH CH_2OH *d*-Glycerose.	CN $\dot{C}H(OH)$ H|OH CH_2OH	CO_2H $\dot{C}H(OH)$ H|OH CH_2OH Trihydroxy butyric acid.	CO_2H HO|H H|OH CO_2H *l*-Tartaric acid.

Hence the spatial formula of *d*-glycerose is established.

Since *l*-tartaric acid is the dicarboxylic acid of *d*-threose the spatial relationship of the dextro-rotatory glycerose to *d*-glucose is also proven.

Fortunately, as pointed out on page 38, the designation of "*d*-glycerose" expresses both its optical activity and spatial relationship to *d*-glucose.

It remains to convert *d*-glycerose into the corresponding monocarboxylic acid by methods which eliminate all possibility of a Walden rearrangement. The formulæ—

$$CH_2(OH).\overset{HO}{\underset{H}{C}}.CO_2H$$

at present ascribed to dextro-rotatory glyceric acid would indicate it is derived from *l*-glycerose. This configuration is based on its preparation from malic acid by Freudenberg, but the reactions may well have involved a Walden rearrangement. It is supported also by the fact established by Frankland, Wharton and Aston that the calcium salt and amide of the acid are lævo-rotatory, indicating by Hudson's rule that the hydroxyl is on the left of the formula, i.e. above the chain of carbon atoms.

The natural pentoses are in reality closely related to the natural hexoses. As the formulæ below show, the arrangement of the groups on the upper four carbon atoms is the same in each case in galactose and arabinose, and the same also in glucose as it is in xylose :—

d-Xylose.	*d*-Glucose.	*l*-Arabinose.	*d*-Galactose.
CHO	CHO	CHO	CHO
HCOH	HCOH	HCOH	HCOH
HOCH	HOCH	HOCH	HOCH
HCOH	HCOH	HOCH	HOCH
CH_2OH	HCOH	CH_2OH	HCOH
	CH_2OH		CH_2OH

In this connection, it is not without interest that some polysaccharides yield both xylose and glucose on hydrolysis, whilst arabinose and galactose occur together in many gums.

When the cyanohydrin synthesis is applied to natural *l*-arabinose a mixture of two nitriles is obtained, and the corresponding acids, when reduced, give rise to *l*-glucose and *l*-mannose ; similarly, *d*-xylose can be converted into *d*-gulose and *d*-idose. *d*-Glucose, when degraded by the methods of Ruff or Wohl, gives *d*-arabinose ; *d*-galactose forms *d*-lyxose. The carbon atom which requires to be eliminated in order that *d*-glucose may give rise to the natural *d*-xylose, a transformation which there is reason to think may take place in the plant, is not the one effected by the processes described, but is situated at the extreme

end of the chain. No chemical means of effecting this change has as yet been discovered.

Arabinose and xylose show the usual aldose reactions. They are not fermented by yeasts. Arabinose forms a characteristic, almost insoluble, diphenyl hydrazone. Xylose is best recognised by conversion into xylonic acid, and isolation of this as the cadmium bromide double salt.

Pentoses are determined quantitatively by distillation with hydrochloric acid when furfuraldehyde is formed. This is coupled with phloroglucinol, and the condensation product isolated and weighed. The colour reactions obtained on heating with orcinol or phloroglucinol and hydrochloric acid are very characteristic, and frequently used for detecting the pentoses.

Hudson finds that xylose can be very readily prepared from cotton-seed hulls with a yield of 8-12 per cent. The hulls are extracted first with 2 per cent. ammonia and then hydrolysed by boiling with 7 per cent. sulphuric acid. The filtrate is carefully neutralised with calcium hydroxide, made just acid with phosphoric acid and concentrated. The remaining calcium sulphate is precipitated on the addition of alcohol and the solution evaporated to a syrup under reduced pressure. This is mixed with alcohol and crystallisation soon takes place.

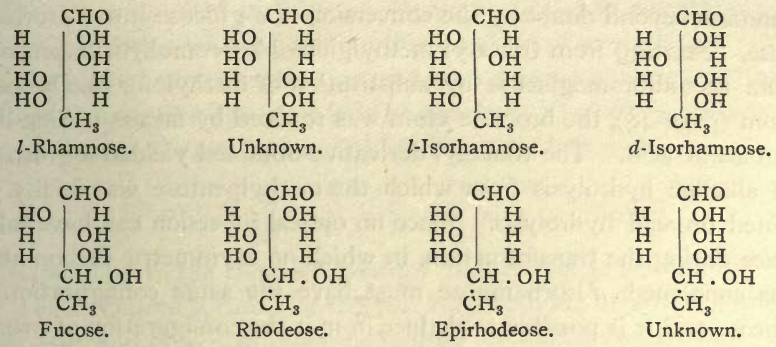
The Methyl Pentoses.

Several representatives of this class of carbohydrates have been discovered latterly in plants. In them, one of the hydrogen groups of the primary alcohol is replaced by methyl. They show most of the reactions characteristic of the pentoses, but form methyl furfuraldehyde on distillation with acids.

Their biochemical significance is not yet understood ; they are not fermented by yeasts. The configuration of most of them has been established by the ordinary methods with the exception of the relative positions of the groups attached to the methylated carbon atom which remain uncertain in some of them, though for rhamnose and isorhamnose this is now established.

The configuration formulæ of the methyl pentoses, so far as at present known, are given in the following table :—

TABLE XII.



Rhamnose, $C_6H_{12}O_5$, is a constituent of many glucosides, the best known of which are quercitrin and xanthorhamnin, the colouring matter of Persian berries. It occurs particularly in combination with flavone derivatives.

Rhamnose crystallises with a molecule of water the hydrate having the composition $C_6H_{14}O_6$; in consequence it was regarded at one time as belonging to the hexahydric alcohols and termed " isodulcitol ".

Rhamnose forms a phenyl osazone and other derivatives similar to those of glucose. It exists in α and β forms which exhibit mutarotation. By the cyanohydrin reaction two rhamnohexonic acids are formed, one of which yields mucic acid when oxidised. The synthesis has been extended to the preparation of rhamnohexose and rhamnoheptose. Methyl rhamnoside is not hydrolysed by enzymes.

In view of the relationship in configuration of rhamnose to *l*-mannose or *l*-gulose it must be regarded as *l*-rhamnose; it is the methyl derivative of the unknown *l*-lyxose.

l-*Isorhamnose* was obtained by Fischer by heating rhamnonic acid with pyridine and reduction of the isorhamnonic acid with sodium amalgam. It is the optical antipode of *d*-isorhamnose (*isorhodeose*), one of the products of hydrolysis of purgic acid, the amorphous constituent of the glucoside convolvulin (Votoček). The crystalline constituent of this glucoside, convolvulinic acid, is hydrolysed to glucose, rhamnose and *rhodeose*. This latter is the optical antipode of *fucose*, which as the polymeride fucosan is a component of the cell-wall of many seaweeds. Votoček has converted rhodeose into *epirhodeose* in the ordinary manner. These compounds and their derivatives have been fully described. The configuration of quinovose, known only in the glucoside quinovin, has not yet been established; other methyl pentoses have been obtained by the hydrolysis of glucosides, which may prove to be new compounds.

Fischer and Zach have established the configuration of these methyl pentoses beyond doubt by the conversion of *d*-glucose into *d*-isorhamnose. Starting from triacetyl methylglucoside bromohydrin, prepared from acetodibromoglucose by substitution of methyl for one bromine atom (page 28), the bromine atom was reduced by means of zinc dust and acetic acid. The triacetyl derivative obtained yielded a glucoside on alkaline hydrolysis from which the methylpentose was finally obtained on acid hydrolysis. Since no optical inversion can have taken place during the transformation, in which no asymmetric carbon atom was concerned, *d*-isorhamnose must have the same configuration as glucose and it is possible to deduce from it the configuration of *l*-rhamnose as that given on the previous page.

Heptoses.

Sugars with seven carbon atoms have now been found to occur naturally so that added interest attaches to the synthetic members of this group.

Mannoketoheptose has been isolated by La Forge from the Avocado pear (*Persea gratissima*). It crystallises in six-sided prisms $[a]_D + 29°$. It is not fermentable by yeast and does not show mutarotation. The phenylosazone is identical with that of *d*-mannoaldoheptose. On reduction with sodium amalgam *d*-perseitol and *d*-β-mannoheptitol are formed.

The following formula is therefore assigned to mannoketoheptose :—

$$\begin{array}{l} \quad H\ \ H\ \ OH\ OH \\ CH_2(OH)\,.\,C\,.\,C\,.\,C\,.\,C\,.\,CO\,.\,CH_2(OH) \\ \quad OH\ OH\ \ H\ \ H \end{array}$$

Perseulose.—The natural alcohol, perseitol, which is also obtained on reduction of mannoheptose is converted on oxidation by *B. xylinum* to a crystalline ketose having a sweet taste and $[a]_D - 81°$. It exhibits mutarotation (Bertrand). On reduction with sodium amalgam two alcohols are formed, perseitol and perseulitol.

La Forge considers perseulose to be *l*-galactoheptose with the structural formula—

$$\begin{array}{l} \quad H\ \ \ OH\ \ OH\ \ H \\ CH_2(OH)\,.\,C\,.\,C\,.\,C\,.\,C\,.\,CO\,.\,CH_2(OH) \\ \quad OH\ \ \ H\ \ \ H\ \ OH \end{array}$$

obviously both mannoketoheptose and perseulose would give rise to the alcohol—

$$\begin{array}{l} \quad H\ \ H\ OH\ OH\ \ H \\ CH_2OH\,.\,C\,.\,C\,.\,C\,.\,C\,.\,C\,.\,CH_2(OH) \\ \quad OH\ OH\ \ H\ \ H\ OH \end{array}$$

which is therefore the formula of perseitol.

The configuration of the heptoses derived from galactose and mannose has been studied by Pierce. Mannoheptitol and galactoheptitol are optical antipodes, each having the structure of perseitol.

The second mannoheptitol derived from β-mannoheptose has three hydroxyl groups on the same side of the chain attached to contiguous carbon atoms, whereas perseulitol has the constitution—

$$\begin{array}{ccccccccccc} & & H & & OH & & OH & & H & & OH & & \\ CH_2(OH) & . & C & . & C & . & C & . & C & . & C & . & CH_2(OH) \\ & & OH & & H & & H & & OH & & H & & \end{array}$$

corresponding to the β-galactoheptitol from β-galactoheptose.

The structural formula of the two ketoheptoses, of α- and β-galactoheptose, and of α- and β-mannoheptose are thus all established.

Sedoheptose was obtained by La Forge and Hudson from a common stonecrop—*Sedum spectabile*—as a syrup. It is non-fermentable, reduces Fehling's solution and gives a phenyl osazone. It is probably a ketose. When it is heated with dilute acid anhydrosedoheptose, $C_7H_{12}O_6$, is produced. This forms crystals, has a sweet taste, has $[\alpha]_D$ − 146° without mutarotation; in boiling dilute acid solution an equilibrium mixture containing 20 per cent. of sedoheptose is formed.

On reduction with sodium amalgam two sedoheptitols are obtained. The one α-sedoheptitol has m.p. 151°, $[\alpha]_D$ + 2·2° in water and + 22·1° in borax solution: it would thus appear to be identical with the natural alcohol volemitol, m.p. 149°-151°, $[\alpha]_D$ + 1·9° and + 2·6° in water and + 20·8° in borax solution.

However, the benzylidene derivatives differ; that from sedoheptitol having m.p. 200° and that from volemitol m.p. 90°, so that the identity of the two alcohols is still an open question. β-Sedoheptitol crystallises in short thick prisms, m.p. 127°-128°, and is optically inactive; the benzylidene derivative has m.p. 272°.

Volemitol, which occurs naturally, is oxidised by *B. xylinum* to a ketoheptose, volemnose, which gives the same phenyl osazone as the reducing sugar obtained by chemical oxidation of the alcohol.

Other Monosaccharides.

Apiose.

Mention may be made of an altogether abnormal sugar, termed apiose, on account of its presence in the glucoside apiin. This contains a branched chain of carbon atoms, having the formula—

$$\begin{matrix} CH_2OH \\ \\ CH_2OH \end{matrix} \!\!\rangle C(OH) . CH(OH) . CHO$$

It is not fermentable, bromine oxidises it to apionic acid. When reduced by hydrogen iodide and phosphorus, *iso*valeric acid is obtained. Apiin contains the disaccharide glucoapiose; when hydrolysed by dilute mineral acids apiose and glucoapigenin are formed.

Cymarose, Digitalose, Digitoxose.

These rare sugars are obtained on hydrolysis of the glucosides cymarin (from the root of apocyanum) and digitalin and digitoxin, two of the glucosides of digitalis.

Digitoxose, $C_6H_{12}O_4$, crystallises in prisms $[\alpha]_D + 46°$. Kiliani has shown it to be a reduced methyl pentose having the following formula :—

$$CH_3 . CH(OH) . CH(OH) . CH(OH) . CH_2 . CHO$$

Cymarose, $C_7H_{14}O_4$, closely resembles digitoxose in behaviour and is considered by Windaus and Hermanns to be a methylether of digitoxose.

Digitalose, $C_7H_{14}O_5$, gives the reactions of an aldose sugar: it is perhaps a reduced methylhexose. All three compounds require further investigation.

The Carbohydrate Alcohols.

Several of the carbohydrate alcohols are widely distributed in plants. They crystallise well and are soluble in water. On cautious oxidation they give in turn a reducing sugar, monobasic acid and dibasic acid. They are not fermentable though attacked by a variety of bacteria and moulds.

Erythritol—$CH_2(OH) . \begin{matrix} OH \\ C \\ H \end{matrix} - \begin{matrix} OH \\ C \\ H \end{matrix} . CH_2(OH)$—is found in many algæ and mosses, particularly *Roccella tinctoria*, where it is present as erythrin, $C_{20}H_{22}O_{10}$, a diorsellinate of erythritol.

It has a sweet taste and is optically inactive, being the *meso* or

internally compensated variety. It has been synthesised from butadiene-γ-δ-oxide

$$CH_2 : CH . CH . CH_2$$
$$\quad\quad\quad

Two heptose alcohols, $C_2H_{16}O_7$, are known, e.g. perseitol, occurring in *Persea gratissima*, and volemitol, discovered in *Lactarius volemus*, and since identified in the rhizomes of some species of primula. Perseitol is the alcohol corresponding to mannoheptose.

An octitol has been isolated from the mother liquors of the sorbitol preparation from the fruit of some of the *Rosaceæ*.

These alcohols are similar in properties to mannitol. Their physical constants are collected below :—

TABLE XIII.

Alcohol.	Melting-point.	Optical Rotatory Power $[\alpha]_D$.
Erythritol	126°	inactive
Adonitol	102°	,,
Mannitol	168°	+ 22·5°
Dulcitol	188°	inactive
Sorbitol	110°	+ 12·3°
Perseitol	180°	− 1·3°
Volemitol	154°	+ 1·9°

Starting from glucose, Philippe has synthesised the higher alcohols of this series, obtaining them by reduction of the corresponding aldoses. *a*-Glucoheptitol is optically inactive and therefore symmetrically constructed. *β*-Glucoheptitol has slight optical activity, the same applies to (*aa*)-gluco-octitol, to (*aaa*)-glucononitol and to (*aaaa*)-glucodecitol, which are therefore all asymmetric in configuration. They are crystalline substances resembling mannitol in their properties.

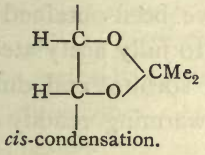
The polyhydroxy compounds can be coupled with acetone or benzylidene residues: fortunately these substances can be methylated by methyliodide in presence of silver oxide without decomposition, and it has thus been possible to study the influence of the position of the hydroxyl groups :—

TABLE XIV.

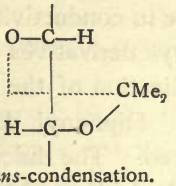
Alcohol.	Number of Hydroxyl Groups Present.	Number of Hydroxyl Groups which React with	
		Acetone.	Benzaldehyde.
Erythritol . . .	4	4	4
Arabitol	5	4	2
Dulcitol	6	4	4
Sorbitol	6	6	4
Mannitol . . .	6	6	6
Glucoheptitol . . .	7	6	2

The series of reactions summarised above shows that the relationship between configuration and condensation is very complex, and that the action of acetone is less irregular than that of benzaldehyde.

Two types of condensation are possible, depending on whether the hydroxyl groups concerned are on the same side or on opposite sides of the carbon chain :—



cis-condensation.

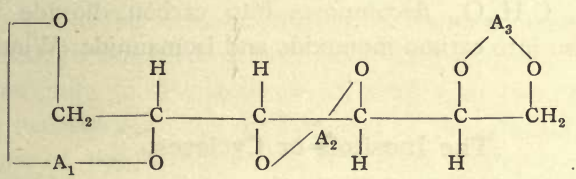


trans-condensation.

It is generally accepted that the ketonic residues are coupled as five-membered rings.

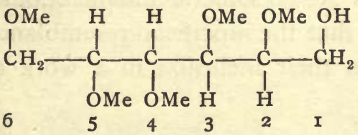
The study of mannitoltriacetone by Irvine and Patterson has shown that it is hydrolysed in stages, both diacetone and monoacetone being formed in turn. The three ketonic residues are symmetrically attached to *a*-carbon atoms, and this difference in stability indicates that the substituents possess the *trans-*, *trans-cis*-arrangement.

The formula of triacetonemannitol is thus—



According to this the terminal alcohol groups in mannitol, though unconnected directly with an asymmetric carbon atom, assume preferentially different positions in virtue of the attractive and repulsive forces exercised by the remaining hydroxyl groups.

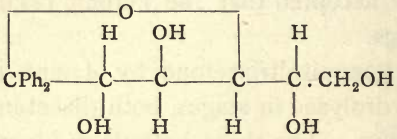
This fact explains the resistance of mannitol to complete alkylation, one of the terminal primary hydroxyl groups remaining unattacked although the other offers no such resistance. The constitution of pentamethylmannitol is—

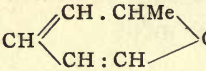


Complete methylation would thus involve the substitution of three adjacent hydroxyl groups on the same side of the carbon chain ; such a process would naturally present difficulties which would not arise were the terminal carbon atom able to take up either position.

According to the older views a di- derivative of mannitol involving groups 1 and 2 would be identical with one in which 5 and 6 were similarly substituted. On the basis of the new formula this would not be the case, but Irvine and Steel have been able to demonstrate for the methyl derivatives that though mannitol contains six reactive hydroxyl groups numbers 1 and 2 are unique, and principally responsible for the increase in conductivity of mannitol in presence of boric acid.[1]

aa-Diaryl derivatives of the alcohols have been obtained by Paal by the application of the Grignard reaction to fully acetylated gluconolactone. Diphenyl, ditolyl and dibenzyl sorbitol and dulcitol are thus prepared. The dulcitol compound on warming readily forms an internal anhydride, probably—



Mannitol has been converted into methyl-*a*-pyrone,  , by treatment with formic acid. The diformate of the mannitan, $C_6H_{12}O_5$, decomposes into carbon dioxide and the pyrone and also into carbon monoxide and isomannide (Windaus and Tomich).

The Inositols or Cycloses.

The molecular formula $C_6H_{12}O_6$ is common not only to the important classes of aldohexoses and ketohexoses, but is shared by certain cyclic polyalcohols such as the hexahydrocyclohexanes. These substances, a number of which are found in nature generally associated with true sugars, contain no carbonyl group and are therefore not, strictly speaking, members of the carbohydrates. On the other hand, they are not only isomeric with the latter but possess a sweet taste, are high-melting crystalline compounds, generally soluble in water, and exist in various stereoisomeric modifications, some of which are optically active; so that the superficial resemblance to the true hexoses is close and justifies their inclusion in a work dealing with natural carbohydrates.

[1] Many sugar and other polyhydroxyl alcohols have their specific rotation increased in the presence of boric acid. Böeseken has shown that such increase is always accompanied by a notable increase in conductivity, and further that this double effect only takes place in compounds in which two hydroxyl groups are attached to neighbouring carbon atoms on the same side of the chain.

Moreover, a perhaps fanciful analogy may be drawn between the hexoses $C_6H_{12}O_6$, the methylpentoses $C_6H_{12}O_5$, and the methyltetrose digitoxose $C_6H_{10}O_4$ on the one hand, and the following series of natural cycloses in which the number of hydroxyl groups are similarly decreased :—

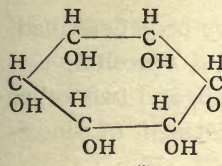
$C_6H_{12}O_6$	the inositols,
$C_6H_{12}O_5$	the quercitols,
and $C_6H_{12}O_4$	of which quinic acid, $C_6H_7(OH)_4 . COOH$, is a carboxylic acid derivative.

If the series is extended to the members $C_6H_{12}O_3$, $C_6H_{12}O_2$ and $C_6H_{12}O$, it becomes clear that these compounds are simply hydrogenated phenols; the less oxygenated members of the group have not been found in nature, but hexahydrophenol, $C_6H_{11}(OH)$, quinitol or hexahydroquinol, $C_6H_{10}(OH)_2$, and phloroglucitol (hexahydrophloroglucinol), $C_6H_9(OH)_3$, have been prepared synthetically by the Sabatier method of hydrogenation of the respective phenols over reduced nickel, whilst quinitol was first synthesised by Baeyer by alkaline reduction of *p*-diketohexamethylene, and phloroglucitol had been similarly prepared from phloroglucinol and sodium amalgam.

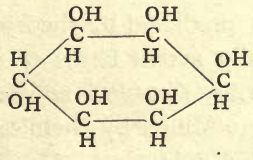
It is of interest that just as glycollic aldehyde is the first sweet tasting sugar in the monosaccharides, so quinitol possesses a sweetish taste, although hexahydrophenol does not; as usual, the presence in close contiguity in the molecule of more than one hydroxyl group appears necessary for the development of this property, at all events in the case of simple compounds of carbon, hydrogen and oxygen.

The stereoisomerism of the cycloses demands some explanation, since it is not due to the simple "atomic" asymmetry of the carbon atoms as in the simple sugars. Thus, in the case of the inositols $C_6H_6(OH)_6$ there are no "asymmetric carbon atoms" of the conventional type, but both dextro- and lævo-rotatory forms of the substance are known; if, however, the steric formula be examined it will be seen that the hydroxyl groups may lie on either side of the plane of the carbon ring-system.

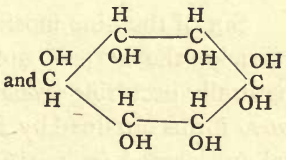
For example we may have :—



(i)



(ii)



(iii)

(i) is a symmetrically arranged molecule identical with its mirror image, but (ii) and (iii) are mirror-images of each other and non-superposable, although it is difficult to show this clearly in a plane illustration. The total number of possible optical isomerides of the inositols may be arrived at by considering the possible number of hydroxyl groups on the "upper" side of the carbon-ring plane in formulæ of of the above type :—

	No. of Hydroxyl Groups.	No. of Forms.
(*a*)	0	1 optically inactive.
(*b*)	1	1 „ active.
(*c*)	2	3 „ inactive.
(*d*)	3	3 „ „
(*e*)	4	3 „ „ (identical with (*c*)).
(*f*)	5	1 „ active (the optical antipode of (*b*)).
(*g*)	6	1 „ inactive (identical with (*a*)).

Of these seven distinct possibilities of optically inactive (*meso*) forms, and two possible enantiomorphic optically active forms, the *d*- and *l*- forms of inositol correspond to configurations (ii) and (iii) in the diagram and to (*b*) and (*f*) in the table, but it is not known to which of the classes (*a*), (*c*) or (*d*), the four inactive or *meso* varieties so far identified, belong.

Similarly, there are ten possible distinct configurations of quercitol, $C_6H_7(OH)_5$, eight optically active and two unresolvable inactive (*meso*) modifications, but only two are known, a dextro-rotatory variety and a lævo-rotatory variety which is not the optical antipode of the former.

The cyclose group may possibly be produced in nature by the union of the ends of the six-carbon hexose chain, but such a transformation has not hitherto been effected in the laboratory.

The only instance of a complex organic compound being obtained from both the ordinary carbohydrates and from the inositols is the formation of furfural when *meso*-inositol is distilled with phosphoric anhydride in a copper vessel (Neuberg).

Inositols, $C_6H_6(OH)_6$.

Six of the nine inositols predicted by theory have been described, namely, the natural optically active forms of *d*- and *l*-inositol, two naturally occurring *meso* forms, *i*-inositol and scyllitol, and two other *meso* forms obtained by Hugo Müller by chemical treatment of *i*-inositol and named *iso*-inositol and ψ-inositol.

No precise configuration is yet attached to any of the inactive forms.

d-*Inositol* is prepared by boiling its naturally-occurring monomethyl ether, *pinitol*, $C_7H_{14}O_6$, with concentrated hydriodic acid; it crystallises in anhydrous prisms which melt at 247°-248° C., and have $[\alpha]_D$ + 65°; it does not show mutarotation.

Pinitol, also known as *matezite* or *sennite*, was discovered in 1856 by Berthelot in the resin of the Californian pine, *Pinus lambertiana* (Dougl.). It also occurs in the residues from the manufacture of coniferin in senna leaves and in Madagascar rubber. Its structure was established by Maquenne.

l-*Inositol* was obtained by Tanret by demethylation of quebrachitol in 1889. It crystallises in needles which melt at 247°, $[\alpha]_D$ − 65°. The monomethyl ether *quebrachitol* is found in quebrache bark.

The racemic inositol, composed of equimolecular proportions of the *d*- and *l*-isomerides, may be prepared by crystallisation of a mixture of the latter in equal quantities, and melts at 253° C. It was found, with *i*-inositol, in the fresh ripe berries of mistletoe by Tanret in 1907.

Meso- or *i*-inositol (*dambose*, *nucite*) is widely distributed in plants and animals; it is found in the muscles and various organs of oxen and horses and in the urine in Bright's disease. In the vegetable world it occurs in the Leguminosae, in the leaves of asparagus, the oak, ash and walnut, and in all parts of the grape vine, and also in many fungi. Its chief sources of extraction are walnut leaves and mistletoe. It crystallises in bunches of needles and melts at 225°. It does not reduce Fehling's solution and is not fermented by yeast, but is attacked by certain fungi. The hexacetate forms monoclinic plates and melts at 212° C.

When *i*-inositol is evaporated almost to dryness with nitric acid and then again carefully evaporated with calcium chloride solution a rose-red solution is obtained; if ammoniacal strontium acetate is substituted for the calcium salt a violet tint is produced (Scherer's reaction). Both these colour tests are excessively delicate, especially the latter.

H. Müller found that treatment of *i*-inositol with a solution of hydrochloric or hydriodic acid in acetic acid transformed it partially into two other *meso* forms:—

iso-Inositol, crystals melting at 246°-250°, readily soluble in water, insoluble in alcohol, but soluble in boiling 50 per cent. alcohol, and tasting faintly sweet; and

ψ-Inositol, an amorphous or microcrystalline compound, very soluble in water, but very sparingly so in alcohol.

The monomethyl ether of *i*-inositol, *Bornesitol*, occurs in Borneo rubber, and the dimethyl ether, *Dambonitol*, in Gabon rubber.

Phytin, a compound of *i*-inositol and phosphoric acid, is an important derivative of *i*-inositol found in the seeds of many plants. It was isolated from rice bran as inositol phosphoric acid by Winterstein and from maize meal by Vorbrodt. It is stable at 115° C., but in presence of water at 155° C. is resolved into phosphoric acid and inositol.

Contardi synthesised an inositol hexaphosphate in 1912 by the action of phosphoric acid on inositol at 120°-130° C. in the absence of air, and considered it to be probably identical with the phytin in seeds.

Anderson at about the same period recorded unsuccessful attempts to obtain the hexaphosphate synthetically, obtaining only tetraphosphates of the formula $C_6H_6(OH)_2O_4[PO(OH)_2]_4$, and inositol derivatives of pyrophosphoric acid.

Scyllitol, the other natural inactive form of inositol, was formerly known by the three names of scyllitol, cocositol and quercine, but H. Müller showed in 1912 that all three were identical.

Scyllitol was discovered by Staedeler and Friedrichs in 1858 in various organs of the spur dog-fish (*Plagiostomi*). H. Müller found it in 1907 in the leaves of *Cocos nucifera* (Linn.) and *Cocos plumosa* (Hook), assigning to it at that time the name "cocositol". It also occurs in acorns.

The formation of the same complex organic product in such different organisms as those of the cocoa-nut palm and oak on the one hand, and the spur dog-fish on the other is very remarkable.

Scyllitol is optically inactive, forms hard lustrous monoclinic prisms which melt at 349°-350° C., is sparingly soluble in water, gives Scherer's colour reaction and yields the customary acetyl, benzoyl, etc., esters.

Quercitols, $C_6H_7(OH)_5$.

As previously mentioned, two optically active forms of the quercitols are found in plants.

d-*Quercitol* occurs in acorns and in small quantities in the cork and bark of oak. H. Müller also obtained it from the leaves of *Chamærops humilis* (Linn.), the only European representative of the palm-family, which was formerly used like esparto for paper-making. The leaves contain 1·35 per cent. of quercitol.

d-Quercitol crystallises in prisms and melts at 234° C.; its rotatory

power is $[\alpha]_D$ + 20°. It is not fermentable. It gives pentacetates and similar esters, thus possessing five hydroxyl groups in the molecule. Oxidation with permanganate leads to the formation of malonic acid and other products which confirm its structural formula as penta-hydroxycyclohexane.

l-*Quercitol* was obtained by Power and Tutin in 1904 from the leaves of *Gymnema sylvestre* (R.Br.). It crystallises in prisms from water and in needles from alcohol and melts at 174° C., having $[\alpha]_D$ − 74°. It gives penta-acetyl and penta-benzoyl compounds, and yields with sodium hypobromite a diketotrihydroxycyclohexane, $C_6H_5O_2(OH)_3$.

d- and *l*-Quercitol are not optical antipodes.

Quinic Acid, $C_6H_7(OH)_4COOH$.

Quinic acid is a carboxylic derivative of a tetrahydroxycyclohexane occurring in cinchona bark, coffee beans, bilberries and other plants. It melts at 162° and is optically active. When it is submitted to dry distillation, phenol, quinol, benzoic acid and salicylaldehyde are produced. Oxidising agents convert it into a variety of common aromatic compounds, notably quinone and hydroquinone. Certain ferments attack it, producing a mixture of the lower fatty acids in absence of air, whilst in presence of air protocatechuic acid is formed.

Quinide, $C_7H_{10}O_5$, melting at 198°, an optically inactive lactone of quinic acid, is produced by heating the natural acid at 220°-240° C., and when this compound is hydrolysed with milk of lime an optically inactive form of quinic acid is produced.

Shikimic Acid, $C_6H_8(OH)_3COOH$.

The only natural representative of the trihydroxycyclohexanes at present known is *shikimic acid*, which is found in the fruit of *Illicium religiosum*, and is an analogue of quinic acid.

CHAPTER IV.

THE DISACCHARIDES.

THE disaccharides or compound sugars are carbohydrates containing twelve carbon atoms and consist of two simple six-carbon atom residues united through an oxygen atom. They are thus analogous to the simple glucosides, and when acted upon by hydrolytic agents—acid or enzymes—they break down by combining with a molecule of water into their constituent simpler hexoses, which may be either aldoses or ketoses :—

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$

One of the constituent hexoses functions in the same manner as glucose does in the methyl glucosides : the aldehydic or ketonic group of the second hexose may remain functional or it may disappear. In the former case the disaccharide reduces cupric salts, forms an osazone, and exhibits mutarotation behaving just as glucose does ; in the latter all these properties are absent. Accordingly, the disaccharides are classified under two types.

The table on opposite page contains the better-known disaccharides with their component hexoses and optical rotatory power. Some trisaccharides are also included ; also the tetrasaccharide, stachyose.

The disaccharides of type I form sparingly soluble phenyl osazones, which are difficult to purify, similar to one another and do not show sharp melting-points as they decompose at the melting-point ; moreover, both melting-point and crystalline form are greatly altered by small quantities of impurities. The hydrazones, even those prepared from asymmetrically disubstituted phenyl hydrazines, are too soluble, as a rule, to be used for the isolation of disaccharides from aqueous solutions.

The difficulty attending research in this group lies in the fact that no really characteristic derivatives of the disaccharides, by means of which they can be isolated and identified with certainty, are known, and partly for this reason but little progress has been made in the direction of their synthesis.

TABLE XV.

Sugar.	Components.	Rotatory Power.
	DISACCHARIDES.	
	Type 1.—*Aldehyde Group Potentially Functional.*	
Maltose	Glucose-α-glucoside	+ 138°
Isomaltose	Glucose-β-glucoside	+ 84·4°
Gentiobiose	Glucose-β-glucoside	+ 9·6°
Cellose	Glucose-β-glucoside	+ 34·6°
Lactose	Glucose-β-galactoside	+ 52·5°
Isolactose	Glucose-galactoside	?
Melibiose	Glucose-galactoside	+ 143°
Turanose	Glucose and fructose	+ 71·8°
	Type 2.—*No Reducing Properties.*	
Sucrose	Glucose and fructose	+ 66·5°
Trehalose	Glucose and glucose	+ 197°
Isotrehalose	Glucose and glucose	− 58°
	TRISACCHARIDES.	
Type 1.		
Mannotriose	Glucose + galactose + galactose	+ 167°
Rhamninose	Glucose + rhamnose + rhamnose	− 41°
Type 2.		
Raffinose	Galactose + glucose + fructose	+ 123°
Gentianose	Glucose + glucose + fructose	+ 31°
Melicitose	Glucose + glucose + fructose	+ 88·5°
	TETRASACCHARIDE.	
Type 2.		
Stachyose	Fructose + glucose + galactose + galactose	+ 148°

Maltose, lactose and melibiose, which reduce Fehling's solution, form hydrazones and osazones with phenyl hydrazine and combine with hydrogen cyanide, contain, like glucose, an aldehyde group or its equivalent. Since they all show mutarotation, and exist in two modifications, there is no doubt that, like glucose, they possess a closed-ring structure rather than a free aldehyde group. In solution they exist as an equilibrated mixture of dynamic isomerides. Both halves of the molecules thus possess a butylene-oxidic structure, one section only retaining the aldehyde group potentially functional.

Interest in the configuration of the disaccharides centres round three main points:—

(1) The nature of the component hexoses.

(2) Whether they represent α- or β-glucosides.

(3) Which hydroxyl group is concerned in the attachment of the two hexose residues?

The solution of the first of these problems is a simple matter. The second question has been answered in two ways: firstly, by studying

the behaviour of the sugar towards the enzymes, maltase and emulsin —if hydrolysed by the former it is an α-glucoside, if by the latter a β-glucoside; secondly, by studying the optical behaviour of the glucose immediately produced, on hydrolysing the sugar with an enzyme, towards a drop of alkali—downward mutarotation classes it as an α-glucose, upward mutarotation indicates the presence of β-glucose. The third question has begun to be solved satisfactorily; and it has been possible to show for maltose, lactose, melibiose and sucrose which groups are concerned in the junction.

Assuming the primary alcohol group to be concerned in the attachment of the two hexose residues four isomeric diglucoses with reducing properties are possible. The attachment of the two glucoses may be either α or β, and the free aldose group will exist in α and β modifications. Maltose or lactose in solution represents, like glucose, an equilibrated mixture of two isomerides: the solid disaccharides correspond to more or less pure single substances.

Considerations based on the numerical relations among the rotatory powers of the disaccharides make it probable that the left-hand glucose residues in lactose and cellose have identical structures, whereas maltose and gentiobiose do not agree with either melibiose or lactose as regards the structure of this glucose molecule.

Three isomerides are conceivable of the non-reducing diglucose according as two α-glucoses, two β-glucoses or an α- and a β-glucose are linked together. These three disaccharides will be single substances either as solid or in solution and they should crystallise more freely than maltose.

The compound sugars of type 2 which contain fructose are regarded as members of the sucrose group since they all contain the sucrose union and are hydrolysed by invertase. Hudson has established that for sugars of this group, which yield fructose and an aldose on hydrolysis, the molecular rotation of the aldose is less than that of its parent sugar by 2340 for its alpha form and 19,300 for its beta form. The specific rotation of the α and β forms of melibiose, gentiobiose and mannotriose calculated in this manner agree with those deduced by other methods.

In the following pages the individual disaccharides are briefly dealt with. The problems connected with their hydrolysis and synthesis are deferred to Chapter VI.

Sucrose.

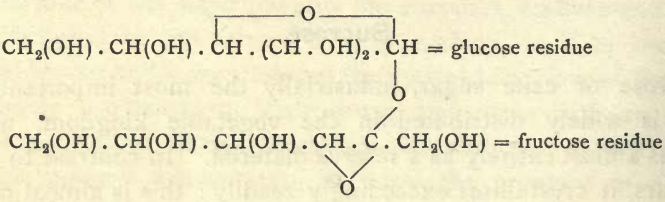
Sucrose or cane sugar, industrially the most important of the sugars, is widely distributed in the vegetable kingdom, where it functions almost entirely as a reserve material. In contrast to most of the sugars, it crystallises exceedingly readily: this is almost certainly due to the fact that a single substance and not a mixture of isomerides is present in solution. It is very soluble in water, and has a much sweeter taste than glucose, but is not so sweet as invert sugar.

Cane sugar does not reduce Fehling's solution nor exhibit mutarotation, and it lacks both aldehydic and ketonic properties. Very characteristic is the behaviour towards mineral acids which hydrolyse it to glucose and fructose. Sucrose is dextro-rotatory, but, since fructose is more lævo-rotatory than glucose is dextro-rotatary, the products of hydrolysis rotate polarised light in the opposite sense to cane sugar. The change in rotation is from $+ 66{\cdot}5^\circ$ to $- 20^\circ$: the process is hence termed inversion, and the product invert sugar. The like change is brought about by an enzyme present in yeasts, moulds, in many plants, also in bees and other animals, and termed invertase or sucrase. Cane sugar is fermented by yeasts only after previous inversion with the invertase of the yeast. Accordingly, it is not fermented by yeasts which do not contain invertase, e.g. *S. octosporus*.

Sucrose forms no compounds with phenyl hydrazine, and is stable towards alkali: this is in marked contrast to the behaviour of the aldoses and ketoses. Sucrose will withstand heating in alkaline solution at temperatures up to 130° without appreciable decomposition. It also does not give rise to glucosidic derivatives. It contains eight hydroxyl groups, as evidenced by the formation of an octa-acetate and an octa-methyl derivative, but gives rise to one form only of these derivatives.

It forms saccharates, $C_{12}H_{21}O_{11}M$, with sodium and potassium hydroxides and more complex saccharates with lime, strontia and baryta.

Until recently it was not possible to ascribe a constitutional formula to sucrose which was entirely satisfactory. It is at one and the same time a glucoside and a fructoside in which the hexose units are joined so as to destroy both aldehyde and ketone groups and give a neutral product. There is much evidence in favour of assigning a butylene-oxide structure to the glucose residue and an ethylene-oxide structure to the fructose residue so that it may be formulated as first proposed by Howarth and Law:—



In the older formula of Fischer, which in turn was a modification of the earlier one of Tollens, a butylene-oxide structure was assigned to both halves of the molecule.

The behaviour of sucrose towards enzymes indicates that it is not a simple glucoside or fructoside; it is not hydrolysed by maltase and the butylene-oxide β-methyl fructoside is not hydrolysed by invertase. Invertase is remarkably active in hydrolysing sucrose, and its action seems to be controlled and inhibited by both glucose and fructose; apparently the enzyme is so constituted that it can adapt itself to both sections of the disaccharide. The question is further discussed in Chapter VI.

The extraordinary instability of sucrose in presence of acids also differs markedly from the behaviour of the simple glucosides, but it is in better agreement with the proposed formula containing an ethylene-oxide ring, as is indicated on page 133.

According to Howarth and Law on hydrolysis of sucrose the following series of changes take place, the final products consisting of the equilibrium mixtures A and C together perhaps with a small proportion of B :—

	A		B		C
Sucrose [→]	α-glucose	+	α-fructose	[⇆]	α-fructose
	↑ ↑		↑ ↓		↑ ↓
	β-glucose		β-fructose	[⇆]	β-fructose
	(Butylene-oxide forms).		(Ethylene-oxide forms).		(Butylene-oxide forms).

The arrows in brackets represent changes involving structure; the others indicate stereochemical interconversions.

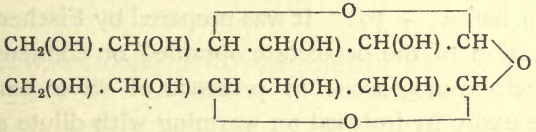
The formula of Howarth and Law is based on the behaviour of octamethyl sucrose on hydrolysis. The optical change is small—from $+66{\cdot}7^\circ$ to $+57^\circ$—whereas had the known butylene-oxide forms of tetramethyl glucose and fructose been obtained an end value of -18° was to be expected. Actually an ethylene-oxide form of tetramethyl fructose having a rotatory power of $+29{\cdot}3^\circ$ is formed. The hydrolysis may thus be formulated :—

Octamethyl sucrose [→] Tetramethyl + tetramethyl
a-glucose *a*-fructose
↓ ↑ ↓ ↑
tetramethyl tetramethyl
β-glucose *β*-fructose
(Butylene-oxide forms). (Ethylene-oxide forms).

The statement that *a*-glucose is a constituent of sucrose is based on the optical changes on hydrolysis of both sucrose and octamethyl-sucrose ; it still requires other confirmation.

Trehalose.

Trehalose, which occurs widely distributed in fungi, is composed of two glucose molecules fused together, so that both aldehydic groups have disappeared :—



This structure is indicated by the fact that it does not reduce Fehling's solution or form a phenyl osazone or exhibit mutarotation. It is not affected by the enzymes maltase, invertase, emulsin or diastase, but is hydrolysed by a special enzyme named trehalase, which is contained in certain fungi and in many species of yeast. Trehalase is conveniently obtained from *Aspergillus niger*. According to Winterstein trehalose is only hydrolysed by acids with considerable difficulty, and contrasts markedly in this respect with sucrose. Presumably the glucose molecules react in the butylene-oxide form.

Trehalose crystallises in lustrous rhombic prisms $[a]_D$ + 197°. The best source for its preparation is *Selaginella lepidophylla* (the resurrection plant) obtainable in large quantities in the arid South-west of America ; this contains 2 per cent. of the sugar which is readily crystallised (Anselmino and Gilg).

Trehalose is also a constituent of sea-weeds. Some of the *Florideæ* contain up to 10 per cent. of the dried material. It is absent from the *Fucoideæ* where it is replaced by a lævo-rotatory disaccharide, termed laminareose, which has not been isolated. Kylin finds that laminarin, the dextrin-like polysaccharide of these plants, consists of a series of closely related substances with a specific rotation varying from − 7° to − 32° as their molecular weight falls. It is hydrolysed by malt diastase to glucose and constitutes a reserve food-stuff analogous

to starch which is utilised by the sea-weed for growth and reproduction during the winter.

Apparently trehalose replaces sucrose in those plants (fungi) which contain no chlorophyll and do not manufacture starch. The quantity of trehalose is a maximum just before the formation of spores. When the fungi are picked the trehalose is rapidly converted into mannitol, being hydrolysed by its enzyme to glucose, which is in some way then reduced. To obtain it, the fungi must be extracted with boiling solvents, so as to kill the enzyme, within two or three hours after gathering.

As indicated on page 144 there are three possible combinations of the two glucose molecules in trehalose. Hudson has calculated the specific rotations of these forms to be $\alpha\beta$ + 70°, $\alpha\alpha$ + 197°, $\beta\beta$ − 58°, and thus identifies the natural sugar as the $\alpha\alpha$ form. It is probable that the $\beta\beta$ form is represented by *iso*-trehalose, which, in an amorphous impure state, had α_D − 39°. It was prepared by Fischer and Delbruck by saponification of the octacetate obtained on condensing two molecules of acetobromoglucose in presence of silver carbonate and is stated to be easily hydrolysed on warming with dilute acids.

MALTOSE.

Glucose-α-Glucoside.

A sugar was first isolated from the products of hydrolysis of starch by De Saussure in 1819, but it was not until 1847 that this new sugar was further examined by Dubrunfaut and named maltose. This discovery seems to have lapsed into comparative oblivion until the sugar was rediscovered by O'Sullivan in 1872. Maltose is prepared by the action of diastase on starch, the only other product of the change being dextrin. It crystallises in minute needles, has a high dextrorotatory power and exhibits upward mutarotation, i.e. the rotatory power when the disaccharide is first dissolved is smaller than the equilibrium value.

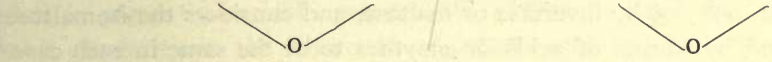
Maltose reduces Fehling's solution, forms a phenyl osazone, and shows many other of the properties of glucose.

When hydrolysed by acids two molecules of glucose are formed. It is very much more resistant to acid hydrolysis than cane sugar.

The enzymes diastase, invertase, lactase and emulsin are without action, maltase alone of all the known enzymes being able to effect hydrolysis. Maltose is fermented only by those yeasts which contain maltase, and then not until inversion has been brought about by the

enzyme. In view of the behaviour of maltose towards maltase, it is considered to be a glucose-*a*-glucoside, since it is only *a*-glucosides which are hydrolysed by maltase; and in confirmation of this view *a*-glucose has been proved to be formed initially on hydrolysis.

Maltose yields, on oxidation with bromine, an acid containing the same number of carbon atoms, which is termed maltobionic acid ; this is hydrolysed to glucose and gluconic acid by mineral acids. Maltose combines with hydrogen cyanide, forming a compound which, on hydrolysis, gives maltose carboxylic acid, and is hydrolysed by mineral acids to glucose and glucoheptonic acid. Maltose must contain eight hydroxyl groups, as it gives an octa-acetyl derivative when acetylated. The behaviour of maltose is in accord with the constitutional formulæ below. As already stated, it is not known which carbon atom is concerned in the attachment of the two sugar residues. Provisionally, the terminal carbon atom is so represented (see Chapter VI.):—



Strong confirmation of this structure is afforded by the behaviour of maltose on oxidation with alkaline hydrogen peroxide, studied by Lewis and Buckborough. Relatively large amounts of glycollic acid glucoside are formed, showing that the primary alcohol carbon atom is concerned in the attachment. The participation of the first, second and third atoms from the free aldehyde group in the glucoside union is precluded by the formation of *a*- and *β*- *iso*-saccharinic acids on oxidation, and the non-formation of glyceric acid glucoside excludes the fourth atom.

Maltose forms a glucoside analogous to methyl glucoside, but the direct condensation with methyl alcohol in presence of acid is not possible, as the disaccharide becomes hydrolysed during the operation. *β*-Methyl maltoside has been prepared from acetochloro maltose, obtained by the action of hydrogen chloride on maltose octa-acetate. Acetochloro maltose interacts with methyl alcohol in presence of silver carbonate, forming hepta-acetyl methyl maltoside, which is converted into methyl maltoside on hydrolysis with baryta. The behaviour of this maltoside towards enzymes is interesting. Maltase hydrolyses it at the *a*-junction, forming glucose and *β*-methyl glucoside ; emulsin attacks only the *β*-junction, forming maltose and methyl alcohol. The maltoside is accordingly *β*-methyl glucose-*a*-glucoside.

The conversion of maltose octa-acetate into *β*-methyl maltoside

fixes it as a β-derivative, and since this acetate is the main product of the acetylation of solid maltose it is probable that maltose belongs to the β series. The rotatory power of crystalline maltose, unlike that of glucose, increases in solution. According to Hudson's rule maltose is a β compound.

Isomaltose.

Isomaltose is the name given by Fischer to the disaccharide obtained by him by the condensing action of strong acids on glucose. It was characterised only by means of the phenyl osazone and the fact that it is not fermented by yeast. Products similar to isomaltose have been repeatedly described as obtained in the hydrolysis of starch, e.g. gallisin, but, failing any characteristic derivative, definite proof of its presence in such cases is lacking. Isomaltose is probably identical with the disaccharide obtained by Croft Hill by the synthetic action of maltase on glucose (see Chapter VI.) which he has termed revertose. E. F. Armstrong has shown that isomaltose is hydrolysed by emulsin, but not by invertase or maltase, and considers the isomaltose obtained by means of acids or enzymes to be the same in each case. The behaviour towards emulsin and maltase suggests that it is probably glucose-β-glucoside.

A quantitative study of the action of hydrochloric acid of much lower strength (0·7 normal) than used by Fischer on glucose has been made by Harrison. He isolated unfermentable isomaltose, α_D + 84·4°, and showed that in 52 per cent. glucose solution the final ratio of isomaltose to glucose is 2 : 3. Davis finds that synthesis of isomaltose takes place in a 1 per cent. solution of glucose in fuming hydrochloric acid (40 per cent. acid).

CELLOSE (CELLOBIOSE.)

Glucose-β-glucoside.

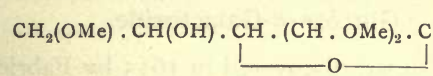
Cellulose (filter paper) when acetylated with acetic anhydride and a small amount of sulphuric acid forms α-cellose octacetate, among other products, from which the corresponding sugar, cellose, is obtained on hydrolysis with alcoholic potash.

Cellose forms a fine crystalline powder, m.p. 225°, and has a faintly sweet taste; it is much less soluble than sucrose. It exhibits mutarotation, reduces Fehling's solution and forms a phenylosazone and osone. Acetochloro, acetobromo and other derivatives are known, cellose behaving exactly like maltose or lactose.

The α-octacetate prepared by acetylation of the sugar in presence of zinc chloride or sulphuric acid has m.p. 229°, $[\alpha]_D$ + 41°. The β-octacetate is obtained on acetylation in presence of sodium acetate and has m.p. 202°, $[\alpha]_D$ − 15°. It is readily transformed into the α-isomeride on heating with acetic anhydride in presence of sulphuric acid, so that the production of α-octacetate by the acetolysis of cellulose does not indicate whether cellulose is a condensation product of α- or β-cellose. Klein has shown that it is possible to obtain 30 per cent. of the theoretical yield of cellose octacetate indicating that at least one-third of the monosaccharides in cellulose are united as in cellose.

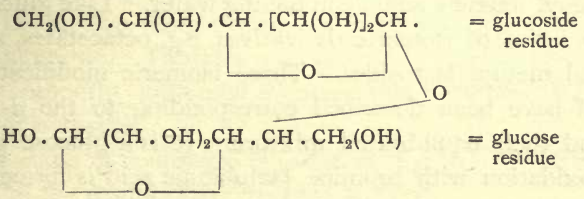
Cellose is not attacked by the enzymes of yeast (maltase). It is hydrolysed by emulsin, and slowly by Kephir lactase and by *Aspergillus niger*. Fischer points out that inasmuch as emulsin is a mixture of enzymes it is not certain that the same enzyme which hydrolyses β-methylglucoside also resolves cellose, gentiobiose and isomaltose. Bertrand and Compton have established the individuality of cellase, the enzyme acting on cellose, and shown that cellase and emulsin occur together in plants in variable proportions. There is, however, considerable similarity in the behaviour of lactose and cellose towards enzymes, and this analogy becomes all the more striking when Hudson's proof that the glucose residues in these two sugars have similar structures is considered.

When methylated cellulose is hydrolysed the most characteristic methylated glucose obtained is a crystalline trimethylglucose. Denham and Woodhouse have established the formula of this as :—



It is definitely a butylene-oxide compound.

Should the same trimethyl derivative be derived from cellose, and taking into account also the similarity between the glucose residues in cellose and lactose, it is possible to suggest the following structural formula for cellose :—



GENTIOBIOSE.

Glucose-β-glucoside.

Gentiobiose is closely allied to maltose, isomaltose and cellose, being composed of two glucose molecules. It is found in the form of a trisaccharide, termed gentianose, present in the roots of various species of gentians; when partially hydrolysed either by means of invertase or dilute acids this yields fructose and gentiobiose. The octacetate is conveniently obtained direct from powdered gentian root (Zemplen), and Hudson has obtained as much as 10 grams per kilo of the dry root in this way.

Gentiobiose shows mutarotation, the α and β forms having rotation + 39° and − 11° respectively and the equilibrium mixture − 9·6°. It forms a phenylosazone, m.p. 160°-170°: other derivatives are the α- and β-octacetates and β-methylgentiobioside which has $[\alpha]_D$ 36°.

It is hydrolysed by emulsin and is therefore a β-glucoside. Bourquelot has prepared and isolated it in a pure state by the action of emulsin on a concentrated solution of glucose, and his observations have been confirmed by Zemplen. It is concluded that gentiobiose and isomaltose are not identical, since whereas the octacetate of the former is readily isolated from very impure products an acetyl derivative could not be obtained from isomaltose syrups.

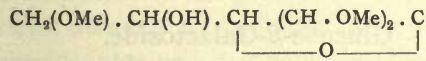
LACTOSE.

Glucose-β-Galactoside.

Lactose or milk sugar, discovered in 1615 by Fabriccio Bartoletti, in Bologna, occurs in the milk of all animals, but has not been encountered in the vegetable kingdom. It is manufactured by evaporation of whey, purified by recrystallisation, and obtained in the form of a white crystalline powder. Mineral acids hydrolyse it to glucose and galactose; it exhibits mutarotation, reduces Fehling's solution, and forms a phenyl osazone soluble in boiling water. Like glucose it gives rise to two series of isomeric derivatives, e.g. octacetates, acetochloro lactoses and methyl lactosides. Three isomeric modifications of the sugar itself have been described corresponding to the α- and β-isomerides and their equilibrated mixture. It is a glucose galactoside, since, on oxidation with bromine, lactobionic acid is formed, and this when hydrolysed by mineral acids gives gluconic acid and galactose, proving that the potential aldehyde group is in the glucose part of the molecule.

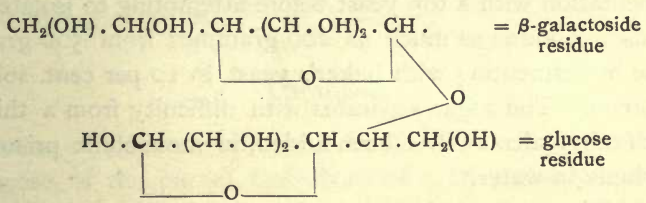
Adopting Fischer's glucoside formula for lactose, it is a question, as previously indicated, whether the primary alcohol group or the δ-secondary alcohol group of the glucose molecule takes part in the union with the galactose. The possibility of either the α- or γ-secondary alcohol groups being concerned is excluded by the facts that lactose forms a phenyl osazone, exhibits mutarotation, and gives rise to derivatives having a butylene-oxide structure. The β-secondary alcohol group can also be excluded from consideration, as Ruff and Ollendorf have obtained, on oxidising the calcium salt of lactobionic acid by Fenton's method, a galactoarabinose sugar which forms a phenyl osazone in which this β-alcohol group is involved. It must therefore be uncombined in the parent lactose.

It remains therefore to decide in favour of the two remaining formulæ. Here again as in the case of sucrose a study of the fully methylated lactose made by Howarth and Leitch enables a decision to be made. Heptamethyl methyl-lactoside, on hydrolysis at 80°, yields tetramethylgalactose and a trimethylglucose having the constitution :—



This affords definite proof that the secondary alcohol group of the fifth or δ-carbon atom of the glucose chain is concerned in the attachment of the two hexose sugars.

Accordingly milk sugar has the constitution :—



The isomeric α and β forms of milk sugar, originally described by Tanret and investigated subsequently by Hudson, differ only with respect to the relative positions of the hydrogen and hydroxyl radicles attached to the carbon atom printed in clarendon type in the glucose half of the molecule. Tanret's γ-lactose is an equilibrated mixture. α-lactose is properly α-glucose-β-galactoside, whereas β-lactose is β-glucose-β-galactoside.

The milk sugar of commerce is α-lactose, $[\alpha]_D$ + 90°. The β form has + 35° and the equilibrated mixture + 55·3°.

Galactoarabinose is of interest as an example of a synthetical disaccharide containing both hexose and pentose sugars. It is

therefore akin to the natural sugar rhamninose. The formation of galactoarabinose affords additional proof that lactose is a galactoside.

Lactose is hydrolysed by a specific enzyme lactase found in a few yeasts (or, more correctly, torulæ), in some kefir preparations, and in the enzyme (crude emulsin) contained in an aqueous extract of almonds. It is believed that kefir lactase and almond lactase are not identical. Lactose is not hydrolysed by maltase, invertase, diastase, nor by any of the enzymes of dried brewers' yeast. Only those yeasts (torulæ) which contain lactase are capable of fermenting milk sugar. Lactose is particularly prone to undergo lactic and butyric acid fermentations.

Isolactose is the name given to a disaccharide obtained by Fischer and Armstrong by the synthetical action of the enzyme kefir lactase on a concentrated solution of equal parts of glucose and galactose, and isolated in the form of the phenyl osazone. It has not been further studied.

MELIBIOSE.

Glucose-β-Galactoside.

Melibiose, together with fructose, is obtained from the trisaccharide raffinose by hydrolysis with dilute acids or certain yeasts (Scheibler and Mittelmeier). It crystallises with difficulty and it is advisable to remove the fructose from the products of hydrolysis of raffinose by fermentation with a top yeast before attempting to isolate it.

Hudson obtains as much as 200 grammes from 500 grammes of raffinose by fermenting with baker's yeast in 10 per cent. solution for 36-48 hours. The sugar separates with difficulty from a thick syrup to which ethyl alcohol has been added, in monoclinic prisms. It is very soluble in water.

It exhibits mutarotation, the α form having + 197° the β form + 125° and the equilibrium mixture + 143°. When hydrolysed with strong acids melibiose yields glucose and galactose. On reduction with sodium amalgam an alcohol, melibiitol, is formed. This, when hydrolysed, is converted into mannitol and galactose. Melibiose is thus a galactoside of glucose, i.e. very closely related to milk sugar.

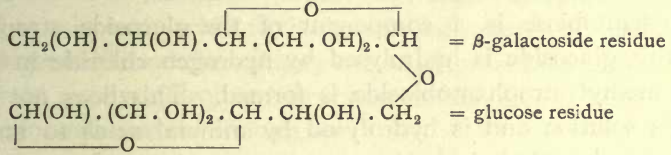
It forms a phenyl osazone, an osone, which latter decomposes to galactose and glucosone and a double series of derivatives in the same manner as lactose.

Melibiose is slowly hydrolysed by emulsin, more rapidly by an enzyme contained in bottom fermentation, but not in top fermentation yeasts: this enzyme is appropriately termed melibiase. Melibiose is

not attacked by maltase, invertase or lactase. It affords a chemical means of distinguishing between top and bottom fermentation yeasts. It is apparently less easily hydrolysed by acids than is milk sugar.

The difference between melibiose and milk sugar appears to depend upon which hydroxyl of the glucose molecule is united to the galactoside (see types A and B, p. 132).

In view of the proof of the structure of lactose afforded by Howarth and Leitch it is highly probable that in melibiose the glucose residue is attached to the galactoside through the terminal carbon of the chain :—



The possibility, however, is not altogether excluded that the third carbon atom from the left in the glucose molecule is concerned.

Added interest attaches to melibiose in view of its being the first natural disaccharide obtained synthetically (Fischer and Armstrong, see p. 143): it was prepared from acetochlorogalactose and sodium glucosate.

Melibiosone, which can be prepared from the osazone by heating with benzaldehyde, is hydrolysed by emulsin or by melibiase to galactose and glucosone.

Turanose.

Turanose was discovered by Alechin in 1890 as a product, together with glucose, of the partial hydrolysis of a trisaccharide, melicitose, with weak acids. He stated that it yielded two molecules of glucose on further hydrolysis, but Tanret subsequently showed that an equimolecular mixture of glucose and fructose is produced. Turanose is hydrolysed with such difficulty that much of the fructose liberated is destroyed by the strong acid solutions that must be used. Turanose is thus an isomeride of sucrose, but differs from this in containing a free aldehydic group, since it forms a phenyl osazone and reduces Fehling's solution. It is said not to exhibit mutarotation and crystallises in colourless rounded grains, $[\alpha]_D$ + 71·8°. It is not at present known whether it is to be regarded as a fructoside or a glucoside. Invertase, maltase, emulsin and diastase are without action.

Vicianose.

Vicianose was obtained by Bertrand from the seeds of a vetch (*Vicia angustifolia*) where it is present in the form of a glucoside, vicianin, allied to amygdalin. Vicianose is glucose-arabinoside, since on oxidation and subsequent hydrolysis gluconic acid and arabinose are formed. Accordingly, in the glucoside the glucose group is attached to the benzaldehyde cyanhydrin.

Strophantobiose.

Strophantobiose is a component of the glucoside strophantin. When this glucoside is hydrolysed by hydrogen chloride in methyl alcohol methyl strophantobioside is formed. This does not reduce Fehling's solution and is hydrolysed by mineral acids to mannose, rhamnose and methyl alcohol.

TRISACCHARIDES, $C_{18}H_{32}O_{16}$.

Mannotriose.

Mannotriose, m.p. 150°, $[\alpha]_D$ + 167°, a colourless faintly sweet crystalline substance, is obtained from stachyose by the action of invertase or of dilute acetic acid. It reduces Fehlings's solution and forms a phenyl osazone, m.p. 122°-124° (Tanret). According to Bierry the compound, m.p. 193°-194°, described by Neuberg and Lachmann was impure. Mannotriose is hydrolysed by acids to glucose (one molecule) and galactose (two molecules). Bromine oxidises it to mannotrionic acid which is hydrolysed by acids to gluconic acid and galactose, thus locating the glucose molecule at the end of the chain. The action of enzymes on mannotriose is still a matter of uncertainty. Bierry has shown that the intestinal juice of the snail probably first forms galactose and a disaccharide, glucose + galactose, which is subsequently hydrolysed. According to Neuberg and Lachmann glucose and a digalactose are formed by the action of almond emulsin.

The constitution is probably

$$\underbrace{\underbrace{CHO.C_5H_{10}O_4}_{\text{Glucose}}-O-\underbrace{C_6H_{10}O_4}_{\text{Galactose}}}_{\text{Gluco-galactose.}}-O-\underbrace{C_6H_{11}O_5}_{\text{Galactose}}$$

Digalactose.

Rhamninose.

Rhamninose, $C_{18}H_{34}O_{14}$, m.p. 135°-140°, $[a]_D$ − 41°, is derived from the glucoside xanthorhamnin present in the Persian berry (*Rhamnus infectoria*). The berries also contain a specific enzyme, rhamninase, which resolves the glucoside into the trisaccharide and rhamnetin. The carbohydrate forms colourless crystals which are somewhat sweet: it reduces Fehling's solution. On hydrolysis by mineral acids galactose and rhamnose (two molecules) are formed. The galactose is proved to be the terminal unit since the rhamninitol and rhamninonic acids, formed by reduction and oxidation respectively, are hydrolysed by acids to dulcitol or galactonic acid and rhamnose (two molecules). Rhamninose is not fermentable and the ordinary enzymes are without action. It appears to be slowly hydrolysed by the intestinal juice of Helix.

The formula may be written:—

$$\underset{\text{Galactose.}}{CHO.C_6H_{10}O_4}—O—\underset{\text{Rhamnose.}}{C_6H_{11}O_3}—O—\underset{\text{Rhamnose.}}{C_6H_{12}O_4}$$

Raffinose.

Raffinose, m.p. 118°-119°, $[a]_D$ + 104°. The best-known trisaccharide is raffinose which is often found in considerable amount in the sugar beet, and is present in other plants. The best source for the preparation of raffinose is cotton-seed meal which contains it to the extent of nearly 8 per cent.: this proportion of the weight of the cotton-seed cake that is produced annually in the United States amounts to 100,000 tons. The raffinose is extracted from the meal with water, and after purification by means of its barium salt, may be isolated from the latter by exact neutralisation of the barium with phosphoric acid. Strong mineral acids hydrolyse it completely to fructose, glucose and galactose in equal proportions. Dilute acids form melibiose and fructose. The action of enzymes on raffinose is more specialised; invertase converts it into fructose and melibiose. Emulsin, however, hydrolyses it to sucrose and galactose. Bottom yeasts which contain both melibiase and invertase are able to ferment it completely.

Raffinose has no reducing action and behaves chemically as cane sugar. The constitutional formula may be written:—

$$\underbrace{\underset{\text{Fructose}}{C_6H_{11}O_5}—O—\underset{\text{Glucose}}{C_6H_{10}O_4}}_{\text{Sucrose.}}—O—\underbrace{\underset{\text{Galactose}}{C_6H_{11}O_5}}_{\text{Melibiose.}}$$

Gentianose.

Gentianose, m.p. 209°-210°, $[a]_D$ + 31·2°-33·4°, is obtained in faintly sweet colourless crystalline plates by extracting fresh gentian roots with 95 per cent. alcohol. It is non-reducing and is hydrolysed by invertase or very dilute acids to fructose and gentiobiose. Some emulsin preparations, in particular extracts of *Aspergillus niger*, convert it into glucose and sucrose (Bourquelot). Stronger acids hydrolyse it to a mixture of fructose and two molecules of glucose having $[a]_D$ – 20·2°. Animal enzymes are without action, but those of molluscs and crustaceæ, particularly of the snail, act firstly to eliminate fructose and then hydrolyse the gentiobiose (Bierry).

The constitutional formula is thus written :—

$$\underbrace{\underset{\text{Fructose}}{C_6H_{11}O_5}—O—\underset{\text{Glucose}}{C_6H_{10}O_4}}_{\text{Sucrose.}}—O—\underbrace{\underset{\text{Glucose}}{C_6H_{11}O_5}}_{\text{Gentiobiose.}}$$

Melicitose.

Melicitose (Melezitose), m.p. 148°-150°, $[a]_D$ + 88·5°, crystallises in rhombic prisms; it is obtained from Briançon manna, the exudation from the young twigs of the larch. It does not reduce Fehling's solution, exhibit mutarotation, or form a phenyl osazone. Dilute acids, e.g. 20 per cent. acetic acid, hydrolyse it to turanose and glucose, the rotation falling to + 63°. Living yeast and enzymes are without action. Stronger acids give rise to fructose (one molecule) and glucose (two molecules). It forms a hendeca-acetate, m.p. 117°, $[a]_D$ + 110°.

Hudson has obtained it in quantity from the manna exuded by the Douglas fir tree, which contains as much as 75 per cent. of the trisaccharide. He also found the sugar in comb honey which the bees had collected from pine trees. It is also present to the extent of 30 per cent. in Turkestan manna.

The constitution may be represented provisionally by the alternative formulæ :—

1. glucose + fructose + glucose.
2. glucose + glucose + fructose.

These would assign to turanose the structure alternatively of a glucoside or fructoside.

TETRASACCHARIDES, $C_{24}H_{42}O_{21}$.

Stachyose.

Stachyose (Mannotetrose, Lupeose) is found in the tubers of *Stachys tubifera*, in ash manna, in the twigs of white jasmine and in the subterranean parts of *Lamium album.*

It is probably identical with lupeose obtained by Schulze from *Lupinus luteus* and *Angustifolius.* It forms lustrous colourless plates, m.p. 167°-170°, $[\alpha]_D$ + 148°, and tastes quite sweet.

Fehling's solution and alkali are without action on it. Acetic acid and the invertase of yeast hydrolyse it into mannotriose and fructose. Sulphuric acid causes complete hydrolysis to hexoses. It is also hydrolysed by the intestinal juice of *Helix pomatia* which first eliminates fructose, then galactose, and finally resolves the gluco-galactoside remaining as described under mannotriose. Animal intestinal enzymes, though they hydrolyse sucrose, are without action on stachyose, the enzymes of molluscs and crustaceæ are also without action. Vintilesco claims to have hydrolysed stachyose completely by the successive action of invertase and almond emulsin. On oxidation with nitric acid, mucic acid is formed.

The formula may be expressed :—

$$\underset{\text{Fructose}}{C_6H_{11}O_5}\text{—O—}\underbrace{\underset{\text{Glucose}}{C_6H_{10}O_4}\text{—O—}\underset{\text{Galactose}}{C_6H_{10}O_4}\text{—O—}\underset{\text{Galactose}}{C_6H_{11}O_5}}_{\text{Mannotriose.}}$$

It forms an insoluble compound with strontium hydroxide and is so easily separated. Tanret has thus isolated it from haricot-beans and the seeds of a number of other leguminoseae.

Crystalline polyamyloses have been obtained from potato starch paste by the action of *Bacillus macerans.* Tetra-amylose, $(C_6H_{10}O_5)_4$, or α-dextrin crystallises in colourless hexagonal plates, $[\alpha]$ + 128°, hexa-amylose or β-dextrin forms rhombic crystals, $[\alpha]$ + 136°. On acetylation, hydrolysis of the dextrins takes place and from the acetyl derivatives formed diamylose and triamylose were obtained. These crystallise in needles and do not reduce Fehling's solution. Rice starch gives similar definite polyamyloses on degradation.

CHAPTER V.

THE RELATION BETWEEN CONFIGURATION[1] AND BIOCHEMICAL PROPERTIES.

PERHAPS the most important, and at the same time the most interesting, chapter in the chemistry of the sugars is that dealing with the alteration in properties brought about by small changes in the stereo-chemical configuration of the carbohydrate molecule. Although the molecular weight and the gross structure of the molecule remain the same, the very slightest modification in the space arrangement of the groups attached to the chain of carbon atoms is sufficient to affect the biochemical behaviour in the most profound manner. How exactly structure is to be correlated with biological behaviour, and how little variation in structure is permissible, will be seen from the following examples.

It has long been known that the optical antipodes of a substance containing an asymmetric carbon atom behave very differently towards biological agents, such as yeasts, moulds, enzymes, or bacteria. The celebrated researches of Pasteur showed, for example, that the green mould, *Penicillium glaucum*, when allowed to grow in solutions of racemic acid, assimilated only *d*-tartaric acid, leaving the *l*-tartaric acid untouched. It was supposed at the time that the mould was unable to attack the *l*-tartaric acid; later investigations suggest, however, that the mould ultimately destroys both antipodes, but attacks one at a very much greater rate than the other, and probably in a different manner.

From a given racemic substance it is possible to obtain sometimes the one and sometimes the other antipode by utilising appropriate organisms. For example, an excess of *d*-mandelic acid is obtained from *dl*-mandelic acid on treatment with *Penicillium glaucum*, whereas when *Saccharomyces ellipsoideus* is used an excess of *l*-mandelic acid is obtained.

[1] By the term configuration is understood the positions of the hydroxyl groups relative to the skeleton chain of carbon atoms. Change involves transference from the right to left side of the chain as figured on the plane of the paper or *vice versâ* from left to right.

Fermentation.

Yeasts only ferment one, the dextro, isomeride of glucose, converting it into carbon dioxide and alcohol, and accordingly when yeasts are allowed to act on racemic glucose the lævo glucose remains unattacked. The same applies to the other fermentable hexoses; in all cases only the dextro isomeride is attacked.

The investigation of the behaviour of all the known hexoses, either found in nature or prepared in the laboratory, towards yeasts has shown that only four are fermented, viz. the *d*-forms of glucose, mannose, galactose, and fructose, all of which are natural products.

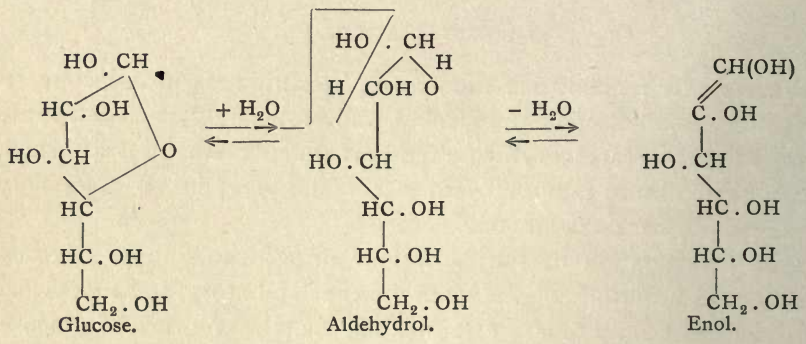
When the behaviour of different species of yeasts towards these natural hexoses is studied, it is found without a single exception that any species of yeast which ferments any one of the three hexoses—glucose, mannose, and fructose—likewise ferments all three of them, and with approximately the same readiness. The study of the kinetics of the three fermentation reactions confirms their similarity, and they have the same temperature coefficient (Slator). Everything, in fact, points to the mechanism involved in the fermentation of glucose, mannose, or fructose being the same in each instance.

It has already been pointed out that the three hexoses in question are closely related in structure, so closely indeed as to be converted under the influence of alkalis into one another. An enolic or oxide form common to all three hexoses has been assumed to act as an intermediate substance in the transformation. The relationship will become clear when the formulæ of these carbohydrates are consulted :—

CHO	CHO	CH . OH	CH_2 . OH
HĊOH	HOĊH	C̈OH	ĊO
HOCH	HOCH	HOCH	HOCH
HCOH	HCOH	HCOH	HCOH
HCOH	HCOH	HCOH	HCOH
CH_2OH	CH_2OH	CH_2OH	CH_2OH
Glucose.	Mannose.	Common enolic form.	Fructose.

It is clearer here to use the older open-chain formulæ, but the reader is advised to study these formulæ in the solid model in order to understand fully the stereoisomerism of these compounds. Representations on a plane surface easily lead to confusion.

On the basis of the closed-ring formula for glucose, enolisation involves in the first place rupture of the pentaphane ring and formation of the aldehydrol; secondly, water is eliminated between two contiguous carbon atoms to give the enol. Comparing the following scheme with that on p. 8, for the conversion of the aldehydrol into glucose, the difference is at once apparent :—



According to the alternative formula the aldehyde forms aldehydrol and this enol. The change is a reversible one.

The process of fermentation of a sugar is regarded as a series of consecutive changes each involving simplification of the sugar molecule till it breaks down into carbon dioxide and ethyl alcohol, compounds containing only one and two carbon atoms. Measurements of the rate of fermentation can be made by determining the rate of formation of either of these products—for example, the amount of CO_2 formed after various intervals of time—but such measurements only apply to the slowest of these reactions. Similarly the quantitative effect produced by an increase of temperature in quickening the rate of fermentation in reality applies to the slowest reaction of the series.

It has been suggested that the first process in fermentation is the conversion of the sugar into the enolic form by means of an enzyme contained in the yeast. The three fermentable hexoses yield the same enolic form, but possibly it is formed at different rates according to the sugar; and whether one and the same agency is operative in each case it is impossible to say. The subsequent simplification of the molecule is the same for each of the three hexoses, an hypothesis which is quite in agreement with the experimental observations. This simplification is also due to an enzyme or to several enzymes acting in turn. The breakdown of the molecule will thus commence at the double linkage between the two terminal carbon atoms.

This view is quite in harmony with the discovery by Harden and Young that the first stage in the fermentation of glucose by zymase is the formation of hexose phosphate $C_6H_{10}O_4(H_2PO_4)_2$. Glucose, mannose, and fructose give rise to the same hexose phosphate: when this is hydrolysed fructose is obtained. In other words, the hexose phosphate may be regarded as a compound of the enolic form of the three hexoses (cp. Dr. Harden's Monograph, p. 46).

Further support of this view of the fermentation process is afforded by the fact that substances so closely related to glucose as the methyl glucosides, glucosone, gluconic acid, and ethyl gluconate are, without exception, unfermentable: in all these only the groups attached to the terminal carbon atom differ from those of glucose. Enolisation in them, however, is impossible, and no action takes place since the formation of hexose phosphate is prevented.

The behaviour of galactose is altogether different. It is fermented with much greater difficulty than glucose. Very many yeasts are quite without action on galactose. The temperature coefficient of the fermentation of galactose is different from the value found in the case of glucose. These facts suggest that galactose is fermented by a different mechanism, that a different enzyme is concerned perhaps in causing enolisation, which is less widely distributed in yeasts. None the less the two phenomena must be very closely allied. No yeast is known capable of fermenting galactose but not fermenting glucose.

The change in configuration in passing from glucose to galactose, though not sufficient to prevent fermentation altogether, causes the compound to be far more resistant to attack. It is not surprising, therefore, that any further change in configuration is sufficient to make the new hexose no longer fermentable.

This is illustrated by the behaviour of galactose and its isomerides, talose and tagatose, which have an enolic form common to all three hexoses:—

CHO	CHO	CH_2OH	CHOH
HCOH	HOCH	CO	COH
HOCH	HOCH	HOCH	HOCH
HOCH	HOCH	HOCH	HOCH
HCOH	HCOH	HCOH	HCOH
CH_2OH	CH_2OH	CH_2OH	CH_2OH
Galactose.	Talose.	Tagatose.	Enolic form.

Neither talose nor tagatose is fermented by any yeast whose action towards them has at present been investigated. Yet in talose the position of the two upper hydroxyl groups is the same as that in mannose, and the lower three hydroxyls occupy the same positions as they do in galactose. Obviously, for it to be fermentable, the configuration of the hexose has to be correct as a whole, the fact that single hydroxyl groups occupy the same positions as they do in fermentable hexoses being of no moment.

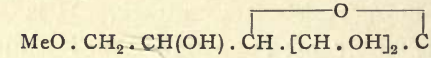
Presumably yeasts contain no enzymes compatible with talose or tagatose and able to convert them into the enolic form.

The facts described can only be explained on the assumption that there is the very closest relationship between the configuration of a

fermentable hexose and the enzymes which cause fermentation. This hypothesis receives confirmation which is little short of absolute when the behaviour of the sugars other than the hexoses is considered. No pentose, either natural or synthetical, is fermentable by yeast. None of the synthetic tetrose, heptose, or octose carbohydrates are fermentable.

The only fermentable sugars, other than the four hexoses, are a *nonose* prepared by the cyanohydrin method from mannose and a ketotriose, *dioxyacetone*. The fermentability of "glycerose"—a mixture of glyceric aldehyde and dioxyacetone—was long a matter of controversy; Bertrand, however, showed tht pure dioxyacetone is fermented by very active yeasts and this has been repeatedly confirmed.

A further illustration of the relation of configuration to fermentability is afforded by the behaviour of that monomethylglucose in which the methoxyl group is attached to the carbon at the extreme end of the chain and therefore most remote from the part of the sugar molecule which is generally believed to have the most effect in controlling enzyme action—



Living top yeast and a maceration extract of dried bottom yeast were quite without action. The compound also resisted seven species of bacteria all of which acted on glucose.

The identification of intermediate products in the fermentation of glucose has long been a matter of controversy.

Buchner and his co-workers have suggested in turn lactic acid $CH_3 . CH(OH) . CO_2H$ and dihydroxy acetone $CH_2OH . CO . CH_2OH$, but in both cases Slator has shown that these are fermented very much more slowly than glucose, an observation which renders Buchner's hypothesis untenable, and the same will probably apply to the latest suggestion that formic acid is an intermediate product. Bearing in mind Fischer's synthesis of acrose from dihydroxyacetone it appears probable that dihydroxyacetone is fermented by yeast only after it has been converted into hexose, and the same applies to glyceraldehyde. This hypothesis is greatly strengthened by Lebedeff's proof that the organic phosphate produced during the fermentation of dihydroxyacetone is identical with the hexose phosphate obtained by Harden and Young from the fermentable hexoses. It is probable, therefore, that dihydroxyacetone is only fermented after conversion into hexose.

Evidence is, however, accumulating that pyruvic acid—

$$CH_3 . CO . CO_2H$$

is a normal intermediary. When yeast is grown in sugar solutions in presence of sodium sulphite considerable quantities of acetaldehyde are formed. This fact is the basis of the suggestion by Neuberg that the sugar breaks down into two molecules of pyruvic acid which are rapidly converted into aldehyde by the yeast carboxylase. The aldehyde in turn acts as an acceptor of hydrogen and promotes the formation of pyruvic acid from sugar under the influence of the yeast reductase, half the aldehyde being at once converted into alcohol. This subject is, however, more appropriately discussed in the monograph on fermentation.

It is obvious how intimately the property of undergoing fermentation is connected with the configuration of the sugar molecule. Lengthening or shortening the chain of carbons is sufficient to place the sugar molecule out of harmony with the yeast enzymes, and thus prevent its destruction by fermentation. The fact that triose, hexose, and nonose sugars are fermentable has led to the suggestion that the fermentable carbohydrates must contain a multiple of three carbon atoms : the fermentability of the nonose requires confirmation.

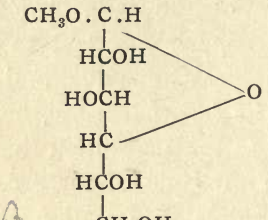
Although hexosephosphate is formed under the influence of yeast juice living yeast cells do not ferment it, even when added coferment and artificial activators are supplied. Dried yeast or yeast juice esterifies phosphate almost quantitatively in presence of sugar whereas living yeast, even when toluene has been added, may esterify only some 8 per cent. ; the difference is probably a question of cell permeability. It is further of interest that some yeasts, when weakened by nitrogen starvation, are able to esterify phosphates in presence of fructose but not with glucose. This is an indication that the protoplasm can grip the ketose structure more readily than the aldose structure and that the preparatory process in fermentation may be concerned in the conversion of aldose into ketose, or far more probably into a common enolic or oxide form, which is more easily formed from fructose than from glucose.

In this connection it is common knowledge that fructose is usually more easily or better utilised in the animal body than glucose, as, for example, under diabetic conditions.

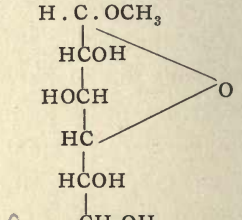
Glucoside Hydrolysis.

The formation of stereoisomeric *α*- and *β*-methyl glucosides by the interaction of glucose and methyl alcohol in presence of hydrogen chloride has already been discussed and their constitutional formulæ established. These isomeric glucosides, though so alike in structure, behave very differently towards enzymes.

α-Methyl glucoside is hydrolysed by the *maltase* (*α*-glucase [1]) of yeast, *β*-methyl glucoside by *emulsin* (*β*-glucase) which is widely distributed in plants. Emulsin is quite without action on the *α*-glucoside; maltase has no effect on the *β*-glucoside.



α-Methyl glucoside hydrolysed by Maltase (*α*-glucase).



β-Methyl glucoside hydrolysed by Emulsin (*β*-glucase).

Other alkyl derivatives of glucose behave in a similar manner. It may be stated as a general rule that *β*-glucosides are hydrolysed by emulsin alone, *α*-glucosides are only attacked by maltase. Accordingly, compounds hydrolysed by emulsin are considered to be *β*-glucosides. The corresponding derivatives of *l*-glucose are not affected in the slightest by either enzyme. *α*- and *β*-methyl-*l*-glucosides represent the mirror images of the methyl-*d*-glucosides and their behaviour is parallel to that of *l*-glucose towards living yeast.

The glucosidic derivatives of mannose, viz. methyl-*d* and *l*-mannosides are also quite stable in presence of maltase or emulsin. Hence the change in position of a single hydroxyl (here that attached to the *α*-carbon atom) is sufficient to render the mannoside out of harmony

[1] *Nomenclature of Enzymes.*—The name of an enzyme is usually derived from that of the sugar which it hydrolyses by substituting the suffix *-ase* for *-ose*. Thus maltase hydrolyses maltose, lactase hydrolyses lactose. The enzyme which attacks glucosides may be termed *glucase* and is an *α*-glucase or *β*-glucase accordingly as it hydrolyses the *α*- or *β*-glucoside.

Although it was at one time generally stated that maltase does not usually occur in plants W. A. Davis gives strong reasons for supposing that it is always present where starch degradation occurs. It is endocellular in origin and readily destroyed by temperatures above 50°: it has low solubility and low powers of diffusion. Daish has identified maltase in the crushed pulp of a number of leaves, all of which convert gelatinised starch into glucose.

with these enzymes; but, as has just been seen, the change in configuration is not sufficient to make mannose unfermentable by yeast.

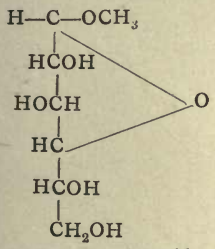
α-Methyl-*d*-galactoside is likewise not hydrolysed by maltase or emulsin.

β-Methyl-*d*-galactoside is hydrolysed by the crude emulsin preparation obtained from almonds, but subsequent investigation has shown that this preparation contains a mixture of enzymes and that the hydrolysis of the *β*-galactoside is due to a lactase (*β*-galactase) and not to the same enzyme which attacks *β*-methyl glucoside. This behaviour shows that the alteration in the position of the hydroxyl attached to the *γ*-carbon atom in the glucoside molecule renders the galactosides out of harmony with maltase and emulsin. Any other alteration involving departure from the configuration of the glucose molecule or in the length of the chain of carbon atoms has the same effect on the behaviour towards enzymes.

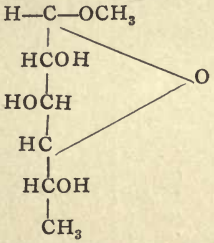
None of the known glucosides[1] of the pentoses, methyl pentoses, heptoses, or other hexoses are hydrolysed by maltase or emulsin.

This behaviour can only mean that the hydrolysing power of these two enzymes bears the very closest relationship to the configuration of the dextro-glucose molecule.

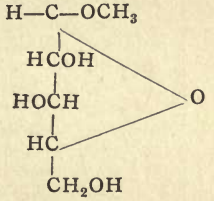
Fischer has drawn particular attention to the behaviour of the *α*- and *β*-methyl-*d*-xylosides. These practically correspond to the corresponding glucosides with one asymmetric carbon atom removed:—



β-Methyl-*d*-glucoside.



β-Methyl-*d*-isorhamnoside.



β-Methyl-*d*-xyloside.

Both xylosides are unaffected by either maltase or emulsin. In this instance, although the major part of the molecule is identically the same in each glucoside, the shortening of the chain is sufficient to destroy the close harmony with the enzyme.

Fischer's latest investigations have shown that *β*-methyl-*d*-isorhamnoside (see p. 121) is also hydrolysed by emulsin. This glucoside differs only from *β*-methyl glucoside in that the terminal CH_2OH group is reduced to CH_3. Apparently such a difference is not enough

[1] The term glucoside is used generally for the corresponding derivatives of all the sugars and not restricted to the derivatives of glucose.

to put the enzyme out of action although, as just stated, the elimination of this carbon atom prevents the enzyme from acting on the methoxyl group at the other end of the chain.

Fischer's own attitude towards this question is expressed in the following extract from his summary in 1898 :—

"Die Indifferenz der Xyloside gegen Emulsin und Hefenenzyme zeigt mithin, welch feine Unterschiede für den Angriff dieser Stoffe massgebend sind, oder mit anderen Worten, wie grob die Vorstellungen noch sind, welche wir trotz aller Fortschrite der Struktur- und Stereochemie von dem Aufbau des chemischen Moleküls haben. Das weitere Studium der enzymatischen Prozesse scheint mir deshalb berufen zu sein, auch die Anschauungen über den molekularen Bau komplizierter Kohlenstoffverbindungen zu vertiefen."

The glucosides investigated by Fischer are summarised in Table XVI. in which + indicates hydrolysis, o denotes no action.

TABLE XVI.

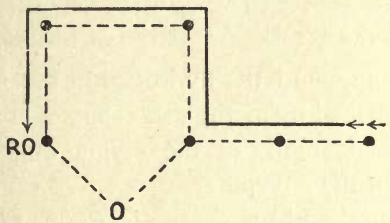
Glucoside.	Maltase (α-Glucase).	Emulsin (β-Glucase).
α-Methyl-*d*-Glucoside	+	o
β-Methyl-*d*-Glucoside	o	+
α-Methyl-*l*-Glucoside	o	o
β-Methyl-*l*-Glucoside	o	o
α-Ethyl-*d*-Glucoside	+	o
β-Ethyl-*d*-Glucoside	o	+
β-Phenol-*d*-Glucoside	o	+
α-Methyl-*d*-Galactoside	o	o
β-Methyl-*d*-Galactoside	o	o
Methyl-*d*-Mannoside	o	o
Methyl-*l*-Mannoside	o	o
α-Methyl-*d*-Xyloside	o	o
β-Methyl-*d*-Xyloside	o	o
Methyl-*l*-Arabinoside	o	o
Methyl Rhamnoside	o	o
Methyl Glucoheptoside	o	o
β-Methyl-*d*-iso-Rhamnoside	—	+

The investigation of the rate of hydrolysis of maltose—an α-glucoside—by maltase has shown that change takes place more slowly in the presence of glucose, indicating that this sugar has a definite retarding influence on the enzyme. Other sugars, e.g. mannose, fructose, galactose, arabinose, xylose are quite without influence on the rate of change, proving that the action of glucose is due not to any concentrating effect but to the specific influence exerted by its configuration. The fact that β-methyl glucoside also acts to retard the hydrolysis of the α-glucoside (maltose) affords the strongest confirmatory evidence of this specific hindrance. Part of the enzyme must combine with

the glucose and so be withdrawn from action. Maltase can apparently combine with β-methyl glucoside though quite unable to hydrolyse it.

In an analogous manner the hydrolysis of β-methyl glucoside by emulsin is controlled only by glucose and α-methyl glucoside, and by no other carbohydrate.

These illustrations, selected from a number of carefully worked-out cases, suffice to show the very intimate relation which exists between enzyme and the substance upon which it acts. This can only be explained by supposing some form of combination between the two. The enzyme, moreover, must fit the glucoside at every point along the chain of carbon atoms, thus :—



The combination may perhaps be compared to the way in which the successive fingers of a glove fit on to a right hand: if the position of any finger be altered it is impossible to fit the glove; further, the glove will not fit on the left hand. Fischer's original simile compared the relationship of enzyme to hydrolyte to that existing between a key and the lock for which it is made, the shape of the key enabling it only to unfasten the particular lock to the arrangement of whose wards it corresponds.

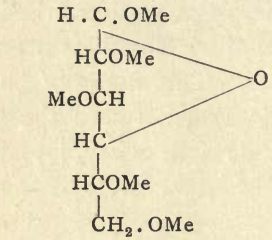
The enzymes themselves, if this hypothesis be accepted, must be closely related in configuration to the substances which they hydrolyse. From this point of view the presence of a carbohydrate in the molecule of invertase and some other enzymes is at least significant (see Monograph by Bayliss, p. 19). Salkowski states, however, that the carbohydrate present in the yeast gum is precipitated with the enzyme, but that it is not a component of the purified enzyme.

It is perhaps necessary to emphasise that the actual hydrolysis of the carbohydrate is due to the action of the water molecules. The enzymes may be conceived perhaps as acting as a vice in presenting in the appropriate manner the water molecule to the centre to be hydrolysed.

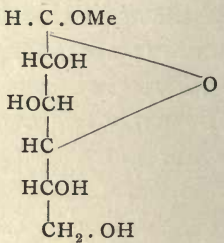
Attachment of enzyme to hydrolyte takes place no doubt through the oxygen atoms of the hydroxyl groups. In these the oxygen atom

possesses residual affinity, that is, is not fully saturated, and it is therefore able to combine with appropriate elements of the molecule of the enzyme.

The fact that tetramethyl-β-methyl glucoside like β-methyl glucoside itself is hydrolysed by emulsin is in full agreement with this view :—



Tetramethyl-β-methyl glucoside.



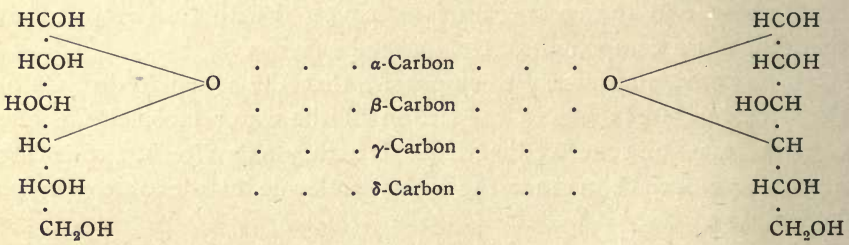
β-Methyl glucoside.

Although in this compound the hydrogen in the hydroxyl groups of glucose has been replaced by methyl, this change is not sufficient either to destroy the residual affinity of the oxygen atoms or to mask them from the influence of the enzyme.

Most of the natural and the synthetic β-glucosides are hydrolysed by emulsin, the exceptions being usually cases where the non-sugar residue is sufficiently toxic to put the enzyme out of action. An interesting exception is afforded by the mandelamide glucosides, of which one only—the lævo form—is hydrolysed, whereas both *d*- and *l*-mandelonitrile glucosides are hydrolysed.

Conversion of Galactose into Glucose.

When the closed-ring formulæ of the two hexoses, glucose and galactose, are considered side by side, it will be obvious that the difference between them is confined to the relative positions of the groups attached to the 4th or γ-carbon atom, i.e. the oxygen atom of the pentaphane ring is attached to different sides of the molecule :—



Glucose. Galactose.

The direct conversion of one sugar into the other involves the rupture of the ring at this point and its closure again in the opposite sense. The whole behaviour of glucose shows, however, that the pentaphane ring ruptures preferentially at the attachment of the oxygen to the first carbon atom. The conversion of glucose into galactose has been only indirectly effected by chemical means, but there is little doubt that it takes place in the organism, as it is only on this supposition that the formation of the galactoside, milk sugar, in large quantities in mammals during lactation can be accounted for.

Under normal conditions the blood transports glucose to the mammary glands, where, in the regular course of lactation, it is converted into the disaccharide, milk sugar, and excreted in the milk. Removal of the mammary gland results in an accumulation of glucose in the blood, from which it passes to the urine. Galactose is not found in the urine. Injection of glucose causes lactosuria when the mammary glands are in full activity, but produces glucosuria when the glands are less active. Nothing is known as to the mechanism by which the mammary glands are able to transform glucose into lactose, but it is undoubtedly effected by means of enzymes.

The enzyme lactase which hydrolyses β-methyl galactoside, other β-alkyl galactosides and milk sugar, is a specific enzyme for β-galactosides, just as emulsin has been shown to be the specific enzyme for β-glucosides. Lactase has its action controlled only by galactose and by no other sugar, and it is incapable of hydrolysing glucosides. The only enzyme at present known which can hydrolyse α-methyl galactoside is the digestive juice of the Helix, which, according to Bierry, attacks both α- and β-galactosides; on the other hand, no compound of α-galactose is known in nature. Bierry states that the lactase obtained from the intestine of a dog hydrolyses lactose and not β-methyl galactoside.

Apparently two lactases exist, one form present in kephir being controlled by galactose, the other present in almond emulsin by glucose. The work of Miss Stephenson indicates that the lactase of the intestinal mucous membrane of animals is a glucolactase.

Oxidation.

The influence of configuration has been also studied in the case of the behaviour of carbohydrates towards oxidising bacteria. The *bacterium xylinum* (Adrian Brown), or sorbose bacterium, as it has been termed by Bertrand, oxidises aldoses to the corresponding monobasic acids, and converts the alcohols into ketones, e.g. gluconic acid is formed from glucose, galactonic acid from galactose; xylose and arabinose yield xylonic and arabonic acids. In all these cases the – CHO group is oxidised to – CO_2H by the agency of the bacterium.

In the case of alcohols the sorbose bacteria oxidise – CH(OH) – to – CO – . Thus mannitol forms fructose; sorbitol yields sorbose; erythritol, arabitol and perseitol are oxidised to the corresponding ketones, and glycerol gives dihydroxyacetone. The bacterium has no action, however, on glycol, dulcitol, or xylitol.

An examination of the formula of these alcohols shows that the – CH(OH) – group oxidised to – CO – is next to a – $CH_2(OH)$ group; further, for action to take place, the hydroxyl group must not be adjacent to a hydrogen atom on the same side of the configuration formula; in other words, the compound must contain the grouping—

$$\begin{array}{l} \\ CH_2(OH) \cdot C \cdot C- \\ \end{array} \quad \begin{array}{l} H\ \ H \\ \\ OH\ OH \end{array}$$

Consideration of the configuration formulæ of mannitol and dulcitol will help to make this clear:—

$$\begin{array}{l} \\ CH_2(OH) \cdot C \cdot C \cdot C \cdot C \cdot CH_2(OH) \\ \end{array} \quad \begin{array}{l} OH\ OH\ H\ \ H \\ \\ H\ \ H\ OH\ OH \end{array}$$

Mannitol—converted into Fructose.

$$\begin{array}{l} \\ CH_2(OH) \cdot C \cdot C \cdot C \cdot C \cdot CH_2(OH) \\ \end{array} \quad \begin{array}{l} H\ OH\ OH\ H \\ \\ OH\ H\ \ H\ OH \end{array}$$

Dulcitol—not attacked.

Gluconic acid contains the sensitive grouping. Accordingly, it is further oxidised by the bacterium to a keto-gluconic acid:—

$$\begin{array}{l} \\ CO_2H \cdot C \cdot C \cdot C \cdot C \cdot CH_2(OH) \\ \end{array} \quad \begin{array}{l} OH\ H\ OH\ OH \\ \\ H\ OH\ H\ \ H \end{array}$$

Gluconic acid.

$$\rightarrow \quad \begin{array}{l} \\ CO_2H \cdot C \cdot C \cdot C \cdot CO \cdot CH_2(OH) \\ \end{array} \quad \begin{array}{l} OH\ H\ OH \\ \\ H\ OH\ H \end{array}$$

Keto-gluconic acid.

In contrast with the sucroclastic enzymes, which are apparently in harmony with the sugar molecule as a whole, these oxidising bacteria seem adapted to a section only of the molecule. Their action is none the less absolutely dependent on the presence of the requisite configuration in the molecule.

Many bacteria act upon mannitol which are without action on

dulcitol. Harden found this to be true for *Bacillus coli communis*, which is of interest also since it produces twice as much alcohol from mannitol as from glucose. This difference is ascribed to the presence of the group $CH_2(OH) . CH(OH)$—which is contained once only in glucose but twice in mannitol.

Only those bacteria which produce fermentation of glucose act on pyruvic acid, $CH_3 . CO . CO_2H$.

According to Grey the fermentation of various carbohydrates and allied substances by bacteria is effected by a single set of enzymes the action of which is common to all such cases of fermentation. The first step in the alteration of a particular molecular structure may require a special enzyme to produce the common intermediate substance but the subsequent changes are always similar, being due to the action of the standard series of bacterial enzymes.

In animal tissues glucose is converted by oxidation into lactic acid with the intermediate formation of glyoxal. Glucosone, which may be regarded as a substituted glyoxal, remains unchanged, however, in presence of a septic kidney tissue, proving that the enzyme can only effect the unchanged hexose (Levene).

A further example of the influence of configuration on biochemical properties is afforded by the formation of the urease ferment by bacteria. Jacoby has shown that whilst *d*-glucose, *d*-galactose and *d* and *l*-arabinose contribute to the formation of the ferment, *d*-mannose and rhamnose are inactive. In the active sugars the configuration—

$$\begin{matrix} OH & H \\ - C & - C - \\ H & OH \end{matrix} \text{ CHO or its optical antipode}$$

exists, whereas in the inactive sugars both hydroxyl groups are on the same side of the chain of carbon atoms.

By floating detached leaves, which have been deprived of their starch by keeping them in the dark, on nutrient solution it is possible to determine which substances can occasion the formation of starch. The application of this method to the carbohydrate alcohols affords an excellent illustration of the influence of configuration on the biological properties. Plants which normally contain alcohols can utilise these and also glycerol to form starch ; thus the *Oleaceæ* utilise mannitol, *Lingustrum* and *Chieranthus* make use of dulcitol. Treboux has shown that the *Rosaceæ* are able to produce starch from sorbitol, the production being more vigorous than from carbohydrates or from glycerol, but they are quite unable to utilise mannitol or dulcitol. The leaves of *Adonis vernalis* are able to convert *adonitol* into starch but can make use of no other carbohydrate alcohols.

The four polysaccharides, sucrose, gentianose, raffinose, and stachyose, may all be regarded as fructose derivatives of increasing complexity. The invertase of beer yeast eliminates fructose from all of them, the juice of *Helix pomatia* or of *Astacus* behaving similarly, though there is a difference in the degree of hydrolysis, sucrose being far the most readily attacked. The intestinal juice of the dog and that of other invertebrates acts only on sucrose (Bierry).

The digestive juice of snails is remarkable in its activity towards substituted lactose derivatives. Thus it hydrolyses lactose-osazone, aminoguanidine, semi-carbazone, and carbamide to galactose and a derivative of glucose. In a similar manner it splits off galactose from derivatives of mannotriose (Bierry).

CHAPTER VI.

HYDROLYSIS AND SYNTHESIS.

Hydrolysis of Disaccharides.

DISACCHARIDES are hydrolysed to monosaccharides by mineral and organic acids in accordance with the equation—

$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6$$

Any acid will act on each sugar, though the intensity of the action differs more or less according to the acid or the disaccharide.

The disaccharides are also hydrolysed by enzymes. The action of enzymes is essentially selective: each particular sugar is hydrolysed only by its appropriate enzyme and by no other. There is thus a sharp distinction between the two classes of hydrolysing agents.

Great historical interest attaches to the phenomenon of the hydrolysis of cane sugar by acids as it was one of the first chemical changes of which the course was followed by physical methods.[1] The change in sign of the optical rotatory power on inversion was first announced by Biot in 1836. A few years later Wilhelmy (1850) showed that the amount of sugar changed in any given moment is a constant percentage of the amount of unchanged sugar present. This is known as Wilhelmy's law, and put into mathematical form it is expressed by the equation :—

$$\frac{dx}{dt} = K(a - x)$$

$$\text{or } K = \frac{1}{t}\log_e \frac{a}{a - x}$$

where $\begin{cases} a = \text{initial amount of sugar.} \\ x = \text{amount already inverted.} \\ t = \text{time which has elapsed since the reaction started.} \end{cases}$

This law has been carefully verified experimentally: the above expression is the simplest type of mass action equation. The velocity constant K represents the rate at which the sugar is inverted.

Cane sugar is hydrolysed at very different rates by different acids. If the acids are classified in order according to their power of hydrolysing sucrose they will be found to be also arranged according to

[1] It is outside the limits of this monograph to do more than indicate the salient features of hydrolysis. A most valuable and complete summary of the literature bearing on the subject, with a bibliography complete up to 1906, is contained in a report presented by R. J. Caldwell to the British Association at York, 1906.

their electrical conductivity and power of hydrolysing methyl acetate. This fact was first recognised by Ostwald in 1884. Other disaccharides and the glucosides are also hydrolysed by acids in accordance with Wilhelmy's law, but hydrolysis takes place far more slowly than in the case of cane sugar. Indeed, whereas cane sugar is rapidly hydrolysed by normal sulphuric acid at 20°, milk sugar requires prolonged heating at 80° to effect the same proportion of change. Armstrong and Caldwell give the relative ease with which hydrolysis takes place as milk sugar 1, maltose 1·27, cane sugar 1240. Other figures relating to the glucosides are given in Table XVII.:—

TABLE XVII.

Compound.	Relative Rate of Hydrolysis.
α-Methyl glucoside	100
β-Methyl glucoside	179
α-Methyl galactoside	542
β-Methyl galactoside	884
Salicin	601
Maltose	740
Milk sugar	582

The relative strength of acids as measured by their inverting power is dependent on the nature of the sugar by means of which the comparison is made, and even with the same sugar the ratio is different at different temperatures. The following table, compiled by Caldwell, illustrates this point. It would, however, lead too far to discuss the significance of these observations here:—

TABLE XVIII.

Sugar Hydrolysed.	Temp.	Relative Activities of the Acids.			
		HCl.	H_2SO_4.	$H_2C_2O_4$.	Camphor-Sulphonic (Reychler).
Sucrose	25°	100	53·7	18·2	89·8
Salicin	95°	100	49·9	23·3	—
Maltose	74°	100	40·5	14·1	—
Lactose	60°	100	47·7	—	68·6
(Conductivity)	25°	100	61·9	19·7	—

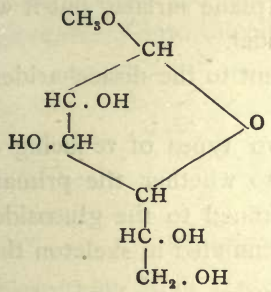
The foregoing data (Table XVII.), though at present somewhat scanty, afford important material for the discussion of the nature of the hydrolytic process. Considering the hydrolysis of the glucosides two views are possible, either (1) that the compound behaves much as the

simple ether $CH_3 . O . CH_3$ would, and that the hydrolyst becomes associated with the oxygen atom to which the CH_3 group is attached ; or (2) that the attachment is to the oxygen atom in the ring. On the former view the two isomeric *a*- and *β*-glucosides should be hydrolysed with equal readiness, as the methoxyl groups are equally weighted in the *a* and *β* position.

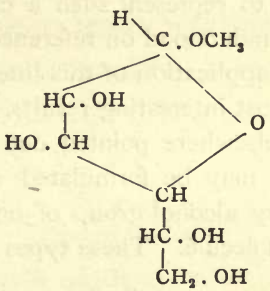
Actually in the case of both glucose and galactose the *β* derivative is hydrolysed about 1·75 times as readily as the *a* derivative, and as there is every reason for thinking that the mechanism of change is the same in both cases, the difference in the rate of hydrolysis can only be due in main to the relative distances of the OCH_3 groups from the centre of the change.

There is little doubt that the active system, within which change takes place, is formed by the association of acid-water molecules with the oxygen atom in the pentaphane ring. Oxonium compounds are formed of the type already discussed at length on pp. 18, 23. In other words, this oxygen is the centre from which attack proceeds.

Reference to a solid model will readily show that a distinct difference exists in the relative distances of the – OCH_3 group, when in the *a* and *β* positions, from the oxygen atom in the ring : this is but imperfectly rendered on a plane surface.



a-Methyl glucoside.



β-Methyl glucoside.

The *a*-methyl glucoside, since it is the most stable form, may be assumed to be that in which the methoxyl (OCH_3) group is furthest removed from the pentaphane oxygen as shown above : conversely, the *β*-glucoside will be that in which the methoxyl is nearest the oxygen centre.

Böeseken assigns exactly the opposite constitutions to *a*- and *β*-glucose, basing his theory on the decrease in the conductivity of *a*-glucose, in presence of boric acid, during mutarotation and the increase in conductivity in the case of the *β* form.

As Irvine has shown, Böeseken, in making his deductions, ignores

entirely the influence of the hydroxyl present as oxonium hydrate for which there is now ample evidence and they may be therefore regarded as invalid. This question has been fully dealt with under the heading Mutarotation on page 15. The same criticism applies to the formulæ for the glucosides suggested by Michaelis based on their acid dissociation constants.

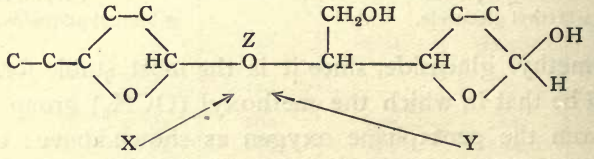
Apparently the rate of hydrolysis of a glucoside can be markedly affected by the nature of the non-sugar residue. In the case of the *a*- and *β*-phenol glucosides Fischer states that under like conditions 68 per cent. of the *a*- and 32 per cent. of the *β*-glucoside were hydrolysed: the corresponding figure for *a*-methyl glucoside being 4·5 per cent. This is the reverse of what obtains with the methyl glucosides. With the menthyl glucosides however, the *β* form is somewhat more rapidly hydrolysed than the isomeride.

The synthetic *a*- and *β*-glucosides will afford valuable material for the complete investigation of these interesting differences.

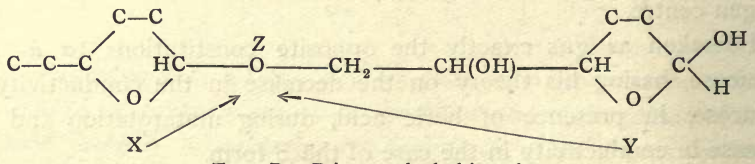
It must be assumed in the case of the galactosides, which are more readily hydrolysed than the glucosides, that the interchange in the position of the groups attached to the *γ*-carbon atom, which involves a shift in the position of the ring, brings the pentaphane oxygen nearer the methoxyl group (p. 13) and so facilitates action. It is impossible to represent such a change on a plane surface, but it will be readily understood on reference to the model.

The application of this line of argument to the disaccharides promises most interesting results.

As elsewhere pointed out (p. 97), two types of reducing disaccharides may be formulated according to whether the primary or secondary alcohol group of one sugar is joined to the glucoside half of the molecule. These types may be formulated in skeleton thus:—



Type A.—Secondary alcohol junction.



Type B.—Primary alcohol junction.

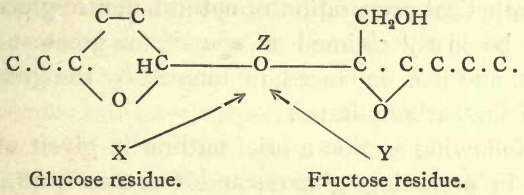
In disaccharides of type A, attack will proceed from both pentaphane oxygen centres X and Y towards the centre marked Z, at which scission of the molecule occurs. Centre Y is further removed from exercising influence than centre X.

In disaccharides of type B, centre Y is still further removed from centre Z, and its influence may be supposed to be correspondingly weakened. Carbohydrates of this type will be least easily hydrolysed.

Differences introduced by the second hexose occupying the α or β positions will mainly affect the distance XZ in the formula, i.e. in practice they will increase or decrease the magnitude of the attack from the centre X, but they will also have an effect on the nearness of the centres Y and Z. As before mentioned, these reasonings are best followed with the aid of a solid model.

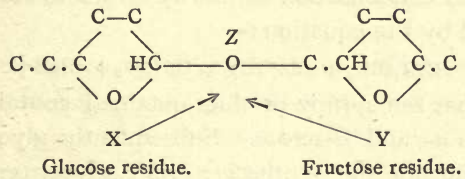
It is possible on the basis of the foregoing argument to assign type formulæ to many of the disaccharides; for example, as lactose is more easily hydrolysed than melibiose it might be assigned to type A and melibiose to type B: other methods have proved this to be actually the case. It is best, however, to defer such speculations until the rates of hydrolysis of all the disaccharides have been compared under comparable conditions, and it is to be hoped, now that many of these sugars are more easily available, that this work will be undertaken.

In cane sugar the ethylene-oxide structure of the fructose residue brings centre Y into the closest possible contiguity with centre Z:—



Everything is in favour of hydrolysis, which, accordingly, may be expected to take place with great rapidity.

In turanose, which is hydrolysed with great difficulty, the centre Y is removed much further away from centre Z, and it may be further assumed that the fructose residue does not in this case possess an ethylene-oxide structure. Conditions are thus opposed to rapid hydrolysis.



It is recorded that isotrehalose is more easily hydrolysed than trehalose: this is in full agreement with the structural formulæ assigned to them.

The laws of hydrolysis by enzymes have been dealt with by Bayliss (Monograph on Enzyme Action), and the details of the selective action towards the disaccharides will be found in Chapters IV. and V. of this monograph.

Enzymes are far more active as hydrolysing agents than acids, a very minute quantity at the ordinary temperature being far more powerful than very strong acid at a high temperature.

It is perhaps desirable here to lay emphasis on the difference noticeable in the behaviour of enzymes and acids respectively as hydrolytic agents. It is due mainly, if not wholly, (1) to the superior affinity of the enzymes for the carbohydrates; (2) to the very different behaviour of the two classes of hydrolysts towards water—which is a consequence of the colloid nature of the one and the crystalloid nature of the other. In other words, whereas there is competition between the solvent water and the carbohydrate for the acid, water has very little attraction for the enzyme: in consequence, practically the whole of the enzyme present is taking part in the operation of hydrolysis.

The Synthesis of Monosaccharides by Chemical Means.

The synthetical preparation of natural dextro-glucose from its elements may be justly claimed as one of the greatest achievements of the chemist, and it is enhanced in interest by the great biological importance of the carbohydrates.

In the following section a brief outline is given of the operations performed in preparing glucose and fructose from their elements. Dealing first with the earlier work, the first attempt which was in any way successful was that made by Butlerow, who showed that when trioxymethylene is condensed by means of lime water a syrupy substance is obtained which has the properties of a sugar. Subsequently Loew improved the technique of the method and named the product he obtained formose. Fischer and Tafel started with acrolein dibromide and effected condensation of this by means of baryta, the change being expressed by the equation:—

$$2C_3H_4OBr_2 + 2Ba(OH)_2 = C_6H_{12}O_6 + 2BaBr_2$$

They showed that the syrupy product obtained contained two sugars distinguished as α- and β-acrose. Subsequently glycerose was made the starting-point for the synthesis; crude glycerose is a mixture of

glyceric aldehyde, $CH_2(OH).CH(OH).CHO$, and dihydroxyacetone, $CH_2(OH).CO.CH_2(OH)$, and these two compounds can be formulated as undergoing the "aldol" condensation forming a ketone, $CH_2(OH).(CH.OH)_3.CO.CH_2(OH)$, which has the same composition as fructose. *α*- and *β*-acrose were obtained from this condensation and characterised by means of the osazones they formed with phenylhydrazine. *α*-Acrosazone was found to possess a remarkable resemblance to glucosazone, differing only in being optically inactive. More recently Fenton has shown that glycollic aldehyde, $CH_2(OH).CHO$, may be used as the starting-point of the synthetical process; three molecules of it condense to *α*-acrose.

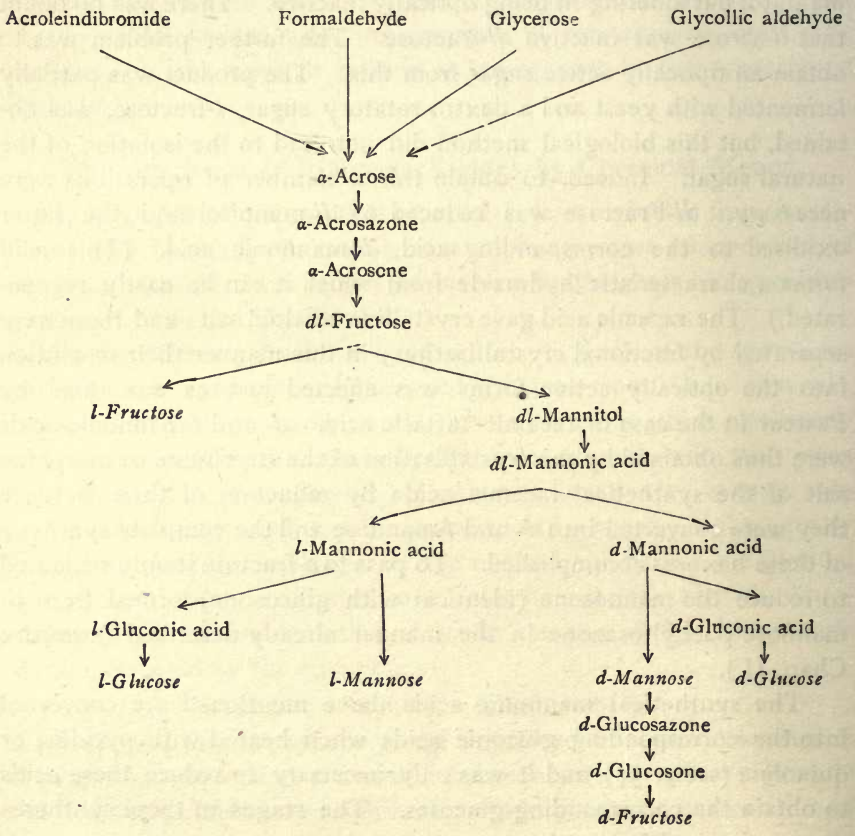
A product of synthesis by all these methods is *α*-acrose. Fischer converted this firstly into acrose phenyl osazone in order to isolate it from the mixture of substances and then into acrosone by treatment with hydrochloric acid as described in Chapter II. Acrosone, on reduction, yielded firstly a sweet syrup having all the properties of fructose, and secondly on further reduction an alcohol, *α*-acritol, very like mannitol but differing in being optically inactive. There was no doubt that *α*-acrose was inactive *dl*-fructose. The further problem was to obtain an optically active sugar from this. The product was partially fermented with yeast and a dextro-rotatory sugar, *l*-fructose, was obtained, but this biological method did not lead to the isolation of the natural sugar. Indeed, to obtain this a number of operations were necessary. *dl*-Fructose was reduced to *dl*-mannitol and the latter oxidised to the corresponding acid, *dl*-mannonic acid. (This acid forms a characteristic hydrazide from which it can be easily regenerated.) The racemic acid gave crystalline alkaloid salts and these were separated by fractional crystallisation; in this manner their resolution into the optically active forms was effected just as was done by Pasteur in the case of racemic tartaric acid. *d*- and *l*-mannonic acids were thus obtained by the crystallisation of the strychnine or morphine salt of the synthetical racemic acid: by reduction of their lactones they were converted into *d*- and *l*-mannose and the complete synthesis of these hexoses accomplished. To pass to *d*-fructose it only remained to reduce the mannosone (identical with glucosone) formed from *d*-mannose phenyl osazone in the manner already described (compare Chap. II.).

The synthetical mannonic acids above mentioned are converted into the corresponding gluconic acids when heated with pyridine or quinoline (see p. 55), and it was only necessary to reduce these acids to obtain the corresponding glucoses. The stages of these syntheses are summarised in the chart on page 136.

Proceeding in this way Fischer effected the synthesis of the six hexoses derived from mannitol, and extended the methods to the synthesis of a number of isomeric hexoses which do not occur naturally. To-day, out of the sixteen possible isomeric aldohexoses, according to the Le Bel-van't Hoff theory, fourteen have been prepared synthetically.

Theoretically a simpler method of passing from fructose (*α*-acrose) to glucose and mannose is afforded by warming with alkali, when the isomeric transformations observed by Lobry de Bruyn take place. These are of particular interest in the case of sorbose, which is converted into galactose and tagatose. Sorbose belongs to the mannitol series, galactose to the dulcitol series, so that this transformation connects the hexoses derived from the two alcohols and indirectly effects the complete synthesis of all the sugars derived from dulcitol.

Before this transformation was discovered Fischer found it necessary to degrade gulonic acid to the pentose sugar xylose, transform this into the isomeric lyxose and combine lyxose with hydrogen



cyanide to give galactonic acid. It was only in this somewhat roundabout fashion that the complete synthesis of galactose and other hexoses derived from dulcitol could be effected.

Fischer regarded the other products of synthesis β-acrose and formose as either allied to sorbose or containing a branched and not a straight chain of carbon atoms. Nef states that formose consists of hexoses and pentoses in equal proportions.

By alkaline condensation of pure glyceraldehyde under conditions which would be unlikely to cause the aldose ⇆ ketose conversion (presence of 0·1 per cent. excess of baryta at the ordinary temperature) Schmitz has obtained a solid crystalline mixture of inactive hexoses. Recrystallisation from hot methyl alcohol separated this into *dl*-fructose (*a*-acrose), m.p. 129°-130°, and *dl*-sorbose, m.p. 162°-163°, which represents β-acrose. The appearance of the ketonic group in the sugar synthesis must take place at the triose stage and therefore, strictly speaking, the reaction is condensation of glyceraldehyde with dihydroxyacetone and not auto-condensation of glyceraldehyde: limitations are thus imposed on the number of hexoses which can theoretically be produced. The mechanism of acrose formation is thus established with a considerable degree of certainty.

Both glycollic aldehyde and dioxyacetone are produced when formaldehyde is condensed by means of calcium carbonate, and H. and A. Euler have shown that a pentose, *dl*-arabinoketose, is the main product of this polymerisation. It is derived from the condensation of glycollic aldehyde and dihydroxyacetone.

$$CH_2(OH).CHO + CO(CH_2.OH)_2 = CH_2(OH).[CH.OH]_2.CO.CH_2(OH).$$

Arabinoketose has not yet been found among plant products.

The Synthesis of Carbohydrates in the Plant.[1]

Though the primary facts of the photochemical assimilation by the green leaf may be regarded as definitely established the full explanation of the process is still outstanding. Priestley (1771), Ingenhouse (1779) and Senebier (1788) established that green plants acquire their carbon from carbonic acid; De Saussure (1804), Boussingault (1861) showed that the volume of oxygen exhaled and that of carbon dioxide absorbed are approximately equal; Sachs in 1862 proved that the first visible product of the process is starch. Brown and Morris (1893) showed that the first sugar which could be identified is sucrose, an observation confirmed by Parkin (1911), and Usher and Priestley (1906) found that

[1] A full account of the historical side of the question has been given by Meldola in a presidential address to the Chemical Society in 1906.

formaldehyde is the first detectable compound of an aldehydic character. Baeyer in 1870 advanced the hypothesis that formaldehyde formed by the reduction of carbon dioxide is the first product of assimilation: the aldehyde is considered to undergo polymerisation subsequently to carbohydrate.

Although this hypothesis is generally accepted as a working basis two difficulties have always been experienced; firstly all attempts to prove the presence of formaldehyde in the green parts of plants have led to inconclusive results, and secondly the experiments made to ascertain whether plants can utilise this aldehyde directly as a source of carbohydrates have indicated that it acts as a poison.

However, more recent investigation now enables both questions to be answered in the affirmative. Usher and Priestley claim to have obtained from leaves, which had been killed by immersion in boiling water, after exposure to light, sufficient formaldehyde to be detected by the usual tests. Their work has been criticised by Ewart, Mameli and Pollacci, but it has been confirmed by Schryver, using Rimini's test for formaldehyde (the formation of a brilliant magenta colour with phenyl hydrazine hydrochloride, potassium ferricyanide and hydrochloric acid). Schryver concludes that chlorophyll can form formaldehyde directly, but that it rarely becomes sensible because it does not accumulate in the cell, since it is withdrawn to form sugars as fast as it is formed.

Boussingault's experiments have been latterly repeated by Willstätter and Stoll in a trustworthy manner, eliminating respiratory effects. They find the "assimilatory quotient," that is the ratio of carbon dioxide absorbed to oxygen liberated, to be unity whether the temperature is 10° or 35° or whether the atmosphere is rich or deficient in carbon dioxide.

Glycollic and glyceric aldehydes and dihydroxyacetone are all intermediate stages in the laboratory synthesis of fructose from formaldehyde, but there is no evidence of these being found among normal plant products. They have so far only been encountered as downgrade products of the action of certain bacteria on mannitol or glucose. Attempts to imitate in the laboratory the formation of formaldehyde from carbon dioxide and water, $H_2CO_3 + 2H_2O \rightarrow CH_2O + 2H_2O_2$, have been numerous, but, if some controversial and very doubtful experiments be excepted, formic acid has been in all cases the sole product of the reduction. However, definite proof of the formation of formaldehyde has been given by Fenton (1907), who has shown that it is formed when carbon dioxide is reduced by means of metallic magnesium.

Brown and Morris, in 1893, working with the leaves of Tropæolum, came to the unexpected conclusion that sucrose is the first sugar to be synthesised by the assimilatory processes. It functions in the first place as a temporary reserve material accumulating in the cell sap of the leaf parenchyma. As assimilation proceeds and the concentration of the cell sap exceeds a certain amount, which probably varies with the species of plant, starch is elaborated by the chloroplasts. This forms a more stable and permanent reserve material than the sucrose. Sucrose is translocated as glucose and fructose, starch as maltose, the latter process only taking place when the starvation of the cell has induced the dissolution of the starch by the leaf diastase. Fructose and glucose are the sugars which contribute most to the respiratory requirements of the leaf cell, glucose being more quickly used up than fructose. Probably a larger amount of fructose than of glucose passes out of the leaf into the stem in a given time.

Parkin selected the leaves of the snowdrop (*Galanthus nivalis*) for investigation since this leaf does not form starch except in the guard cells of the stomata, though the bulb contains starch and inulin in abundance. Maltose was also proved to be absent from the leaf. His analyses confirm Brown and Morris that sucrose is the first sugar to appear and that the hexoses arise from it by inversion. Here again the quantity of fructose in the leaf is almost invariably in excess of that of glucose. The total quantity of the hexoses remains remarkably constant.

Although the weight of evidence is strongly in favour of the view that sucrose is the first sugar formed in photosynthesis, some observers hold that hexoses are to be regarded as the primary products, sucrose being formed later by synthesis either in the leaf or in the root. Strakosch, for example, employing microchemical methods, concluded that glucose was the first sugar formed in the mesophyll of the leaf: his experimental work does not, however, carry conviction. In the previous edition of this book attention was directed to the work of Campbell but it has since been shown by Davis that in Campbell's analyses the cane sugar was greatly under-estimated, and his work must therefore be regarded as merely preliminary and his data and conclusions entirely withdrawn.

A most careful study of the carbohydrates of the mangold leaf under actual normal conditions of growth has been made by Davis, Daish and Sawyer in 1916, whose papers summarise the work in this very difficult field. The facts they bring forward confirm the view of Brown and Morris that sucrose is the primary sugar formed in the

mesophyll of the leaf under the influence of the chlorophyll. It is transformed into hexoses in the veins, midribs and stalks, the proportion of hexoses increasing as the root is approached. It enters the root as hexose and is there reconverted into sucrose, remaining as such until required for the growth of the second season. Invertase is entirely absent from the root, so that it is highly improbable that the synthetic change is effected by this enzyme.

Starch is entirely absent from the leaf after the very earliest stages of growth, and maltose is entirely absent at all stages and at all times. Pentoses only form a small proportion of the total sugars; they are apparently formed from the hexoses and appear to be precursors of the pentosans.

Davis shows that the determination of glucose and fructose separately in leaf extracts is rendered difficult by the presence of optically active impurities not precipitated by basic lead acetate: it is therefore premature to draw any conclusions from the proportion of apparent glucose or fructose in plant tissue as to whether either of these sugars is better adapted than the other to tissue formation or to respiration. Davis, however, considers that the two hexoses exist in the leaves and stalks as invert sugar and travel in nearly, if not exactly, equal proportions to the root.

Davis has studied in a similar manner the fluctuations in the carbohydrates in the potato leaf. Here also the first sugar to develop is sucrose: its amount increases from sunrise up to 2 P.M. and then falls. Up to 2 P.M. there is very little fluctuation in the amount of starch. At 2 P.M. the hexoses (derived from sucrose by hydrolysis) begin to increase, soluble starch appears for the first time and its amount increases regularly up to a maximum at 6 P.M. Between this hour and midnight the amount of starch falls so that finally only a very small proportion is left (0·2 per cent.). The starch is apparently converted directly into glucose, the amount of which increases in the leaf. Maltose is invariably absent from the potato leaf and also from the leaves of other plants which form much starch in the leaf; apparently the leaf enzymes are able to degrade starch completely to glucose. Glucose is certainly in excess in the stalks of the potato, and the starch in the tuber is built up from this hexose.

Assuming that Baeyer's hypothesis is correct and that formaldehyde is the first product of the synthesis, two questions await an answer. Firstly, how is the condensation of the aldehyde caused; secondly, through what intermediate stages do the compounds pass?

The vital synthesis differs essentially from that carried out in the

laboratory in affording optically active products. It might be supposed that the plant manufactures inactive racemic hexose and uses the lævo-isomerides for purposes which are still unknown. In spite of frequent search, however, it has never been possible to detect *l*-glucose or *l*-fructose in the leaves of plants, and the work of Brown and Morris leaves hardly any doubt that hexoses of the *d*-series and their polysaccharides are the only products of assimilation.

The living organism is not satisfied with merely elaborating a particular sugar, but shapes it in a definite manner to a definite space configuration.

Fischer has pictured the carbon dioxide or formaldehyde as entering into combination with the complicated optically active protoplasm of the chlorophyll granule, and being synthesised to optically active carbohydrates under the influence of the asymmetry of the protoplasm molecule.

The formaldehyde elements are received one after the other, and superposed according to a definite plan until six are united, when the completed dextro-glucose or fructose molecule is split off and the process begins anew, only optically active substances being formed. Synthesis by laboratory methods leads to optically inactive forms, though apparently chemical synthesis does not take place entirely symmetrically when several asymmetric carbon atoms are present.

It is now generally agreed that the protoplasm of the chlorophyll granule contains enzyme elements, and that it is these which occasion synthesis. The protoplasmic complex may be regarded as built up of a series of associated templates (enzymes) which serve as patterns for the maintenance of vital processes and of growth. The assimilated carbon dioxide, either before or after condensation to formaldehyde, is brought into contact with these templates in the protoplasm, and contiguous molecules are united to form the complete sugar, shaped according to the structure of the template. The enzyme specific for each particular hexose when incorporated in the protoplasmic complex may well serve as the template for its manufacture. Maltase, for example, might occasion the formation of *α*-glucose, emulsin that of *β*-glucose, lactase that of galactose, and invertase, or some similar enzyme, that of fructose. The existence of contiguous maltase and invertase[1] branches in the protoplasmic complex might determine the

[1] Armstrong's recent researches suggest that invertase is compatible, at one and the same instant, with both glucose and fructose, so that its presence in the protoplasmic complex would, under suitable conditions, lead to the formation of cane sugar. It is probable that invertase is only compatible with the ethylene-oxide form of fructose and not with the butylene-oxide isomeride.

formation of glucose and fructose in contiguity, and these might unite to cane sugar. Again, two glucose molecules in contiguity might unite to maltose, or a series formed in contiguity might remain potentially active so that a number would unite and give rise to a starch molecule. α- and β-glucose would remain as such so long as they were incorporated with the protoplasm; when split off into the cell fluid they would no doubt tend to pass over in the equilibrated mixture.

Certain claims have been made in reference to the synthesis of carbohydrates from simple substances by means of sunlight or ultraviolet light. Thus glycerol in alkaline solution is partly converted into α-acrose (Bierry and Henri) after exposure to ultraviolet light; after many months in sunlight sorbose has been obtained from a mixture of formaldehyde and oxalic acid (Inghilleri).

The Synthesis of Disaccharides.

Although in the hands of Fischer the problem of the synthetical preparation of the natural simple carbohydrates—the monosaccharides—has been solved, the next step, the synthesis of the disaccharides, still awaits a satisfactory solution.

The earliest synthetical disaccharide was obtained by Fischer by the action of cold concentrated hydrochloric acid on glucose. The compound obtained was termed isomaltose on account of the resemblance to maltose, from which it differed in being nonfermentable. The process had the disadvantage that it could not be controlled, so that only small quantities of disaccharide were formed together with considerable quantities of dextrin-like products. It was shown subsequently, as described later, that both maltose and isomaltose are formed by this process. A more hopeful method, based on Michael's glucoside synthesis, appeared to be the combination of acetochloro glucose with the sodium salt of a hexose. This method has been repeatedly used in attempting to synthesise cane sugar, and Marchlewski claimed to have been successful in artificially obtaining this sugar. Subsequent workers have found it impossible to confirm his results, and they are to be queried also for other reasons, chief of which is the observation of Fischer and Armstrong that α-compounds of glucose in presence of alkali undergo rearrangement to β-compounds. These observers failed to prepare α-phenyl glucoside from α-acetochloro glucose and sodium phenolate, obtaining instead the β-phenyl glucoside. Sucrose, a derivative of α-glucose, should not therefore be formed. The evidence brought forward by Marchlewski in proof of the formation of cane sugar was also very inadequate. There are thus no grounds for accepting this synthesis.

By the interaction of acetochloro galactose with sodium glucosate or of acetochloro glucose with sodium galactosate, Fischer and Armstrong obtained disaccharides of the type of maltose which they termed galactosido-glucose and glucosido-galactose. These sugars were sufficiently closely related to the natural products to be hydrolysed by enzymes. Top yeast was without action, bottom yeast was able to ferment both disaccharides. They were hydrolysed by emulsin, but not affected by maltase or invertase. Both reduced Fehling's solution, formed phenyl osazones and osones, but could not be obtained in a crystalline state. The galactosido-glucose possessed very great similarity to the natural sugar melibiose both in structure, similarity of the phenyl and bromophenyl osazones and in physiological behaviour, and it is very probable that these disaccharides are identical.

The galactosido-galactose obtained by the same method resisted the action of yeast but was hydrolysed by emulsin. It is of interest now that crystalline galactobioses have been synthesised by means of emulsin.

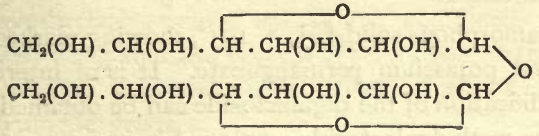
Fischer and Delbrück have made use of β-acetobromo glucose to effect the synthesis of disaccharides allied to trehalose. When acetobromo glucose is shaken in dry ethereal solution with silver carbonate and traces of water are added from time to time, bromine is eliminated and two molecules are joined through the intermediary of an oxygen atom to form an octacetyl disaccharide :—

$$2C_{14}H_{19}O_9Br + H_2O = C_{28}H_{38}O_{19} + 2HBr$$

This is obtained both crystalline and in an amorphous form, the latter being regarded as a mixture of isomerides.

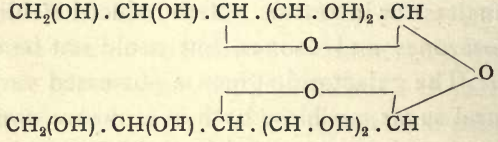
These acetyl compounds when hydrolysed by cold barium hydroxide solution are converted into disaccharides. That from the crystalline acetate, termed isotrehalose, differs from trehalose in optical rotatory power, $[a]_D - 93{\cdot}4°$, but resembles it closely in chemical properties. It is a colourless amorphous powder, which does not reduce Fehling's solution and is easily hydrolysed to glucose when boiled with dilute mineral acids. The disaccharide from the amorphous acetate is regarded as a mixture, it has $[a]_D$ about $- 1{\cdot}3°$. It is remarkable in being partially hydrolysed both by yeast extract and by emulsin.

Consideration of the constitutional formula of trehalose—



shows that three stereoisomerides are possible, as the two carbons in clarendon type are asymmetric. Using the prefixes α and β in the same sense as in the acetobromo glucoses, these isomerides may be described as $\alpha\alpha$, $\beta\beta$ or $\alpha\beta$, according as the constituent glucoses are present in the α or β form.

Hudson from optical considerations has identified natural trehalose as the $\alpha\alpha$ form and isotrehalose as the $\beta\beta$ form :—



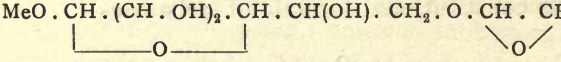
The same method has been extended by Fischer to the synthesis of non-reducing tetrasaccharides from acetobromo lactose and acetobromo cellobiose. In both cases the products were contaminated with reducing disaccharide and they could not be purified.

The isotrehalose synthesis has been extended to the preparation of disaccharides containing sulphur and selenium (Schneider and Wrede).

Treatment of β-bromoacetylglucose with potassium hydrosulphide gave the octa-acetate of the thiodisaccharide $C_{12}H_{22}O_{10}S$, which latter crystallises in hexagonal leaflets, m.p. 174°, $[\alpha]_D$ − 85°. It is called thioisotrehalose from its apparent analogy with isotrehalose. It is remarkably resistant to hydrolytic reagents, warm aqueous alkalis and mineral acids. It is unattacked by emulsin, yeast enzyme, trehalase and myrosin. It does not reduce Fehling's solution.

Similarly, from β-bromoacetylglucose and potassium selenide, an octa-acetate of selenoisotrehalose, $C_{12}H_{22}O_{10}Se$, was obtained. The sugar closely resembles the thio-derivative, and melts at 193°, $[\alpha]_D$ − 84°. Both sugars are sweet, and when administered to dogs or guinea-pigs are excreted unchanged.

The remarkable reactivity of the ethylene-oxide form of the hexose sugars is illustrated by their behaviour towards methyl alcohol containing 0·25 per cent. of hydrogen chloride, studied by Cunningham. In the case of galactose the active sugar condenses with the methylgalactosides to form a methyldigalactoside having the structure :—



This is an amorphous solid, $[\alpha]_D$ + 101°, showing marked instability towards neutral potassium permanganate. It is of interest that no structural modification of the digalactoside can be obtained by varying the form of the methylgalactoside employed in its preparation.

The reaction cannot be readily controlled, and when a hexose is dissolved in the methyl alcohol reagent and the solution concentrated, products of greater complexity, viz. a methyltetragalactoside, containing three ethylene-oxide linkages, are obtained. Glucose and maltose behave similarly to galactose, but with fructose charring inevitably occurred on concentration. It is remarkable that lactose remained entirely unaffected by the reagent.

Nef has effected the synthesis of polysaccharides from hexoses and pentoses. By keeping concentrated aqueous solutions at the ordinary temperature in the presence of 1 to 3 equivalents of calcium acetate bishexoses $(C_nH_{2n}O_n)_2$ are slowly formed.

In the presence of metallic hydroxides these are converted into disaccharides ($C_{12}H_{22}O_{11}$) by salt formation and subsequent loss of metallic hydroxide. The synthetic sugars have not been further characterised.

Synthesis by Enzymes.

Far more interesting than the above method of synthesis is that effected by means of enzymes. There can be no doubt that, in the plant, enzymes function as synthetical agents.

The first to observe the synthetical or, as he termed it, reversible action of enzymes was Croft Hill. Hill proved that the hydrolysis of maltose by dried yeast extract in concentrated solutions was not complete, and that, starting from glucose alone in concentrated solution, a disaccharide was produced by the action of maltase. This sugar he at first considered to be maltose, a conclusion controverted by Emmerling, who, repeating Croft Hill's experiments, considered the product to be *iso*maltose identical with that obtained by Fischer by the action of acid on glucose. Subsequently Croft Hill admitted the chief product to be an isomeride of maltose, but he regarded it as different from isomaltose and termed it revertose. He still claimed that maltose is also formed in small quantity. E. F. Armstrong considered that the product of the synthetical action of maltase on glucose was *iso*maltose identical with that produced by the action of hydrochloric acid on glucose, and showed that the two products agree in being hydrolysed by emulsin though not by maltase. They were accordingly regarded as having the structure of glucose β-glucosides. Croft Hill showed that his synthetical product was almost completely hydrolysed on dilution, indicating that the process is reversible, or that at all events the same mixture of enzymes which effects synthesis is able to hydrolyse the synthetic product.

A disaccharide is also formed when a mixture of glucose and galactose in concentrated solution is left in contact with lactase. This is undoubtedly isomeric with milk sugar but differs from it in being completely fermented by bottom yeast.

The process by which a monosaccharide is converted into a disaccharide in presence of a synthetical catalyst must be regarded as precisely similar to that by which α- and β-glucoses are converted into the two methyl-glucosides. Glucose on condensation should give rise to both maltose and isomaltose synthesised from α- and β-glucose respectively. The proportion of each ultimately present in equilibrium will depend to some extent on the proportions of the two glucoses in their equilibrated mixture and on their (possibly unequal) rates of condensation. This reasoning should apply so long as the condensation is uncontrolled. Inasmuch as hydrolysis under the influence of enzymes is an absolutely selective process, as opposed to hydrolysis by acids which is general in character, it is to be supposed that synthesis under the influence of enzymes is likewise a controlled operation.

The proof that hydrochloric acid forms both *iso*maltose and maltose from glucose was first given by E. F. Armstrong. The method of purification of the synthetical isomaltose mixture adopted by Fischer, viz. fermentation of the neutralised product with brewers' yeast, would have destroyed any maltose which had been formed. Armstrong fermented a portion of the product with *S. Marxianus*, a yeast which does not contain maltase and therefore is without action on maltose, in order to destroy the unchanged glucose. The resulting solution contained both maltose and *iso*maltose, and was partially hydrolysed by both maltase and emulsin. To remove the *iso*maltose it was submitted to the joint action of emulsin and *S. Marxianus*. It was not found possible to obtain the maltose in a crystalline condition from this solution, but the character of the osazone formed and the biological behaviour of the sugar leave little doubt of the presence of this sugar. Another portion of the original synthetical sugar was fermented with *S. intermedians*, and so freed from glucose and maltose. The resulting *iso*maltose solution behaved in all respects as described by Fischer.

The manner of the synthesis by enzymes is still a matter of dispute. It is urged, on the one hand, that enzymes produce by synthesis the same bodies which they hydrolyse; on the other hand, it is suggested that the action of the enzyme is restricted to the formation of a compound isomeric with that normally hydrolysed by the enzyme. A third view is that altogether distinct enzymes effect synthesis.

The arguments in favour of accepting the first view have been clearly put by Bayliss (see the Monograph on Enzyme Action in this series), and need not be repeated here.

The question is complicated by the fact that the catalysts used are all mixtures of several enzymes. Yeast extract (maltase) contains at least five sucroclasts; emulsin at least three.

Armstrong has shown that the main product in the case of the action of yeast extract on glucose is isomaltose; and contended that in the case of emulsin the main product is maltose.

This contention can no longer be maintained in view of the proof given by Bourquelot and confirmed by Zemplen that gentiobiose is the product of the condensation of glucose in presence of emulsin. They have isolated the sugar in a crystalline state, and to Bourquelot belongs the credit of the first synthesis of a crystalline natural disaccharide.

In all the above syntheses it cannot definitely be asserted that other isomerides are not also formed. When the complexity of the enzyme mixture, the number of reactive forms of the hexose and the variety of possible isomeric disaccharides are all taken into account the magnitude of the problem of their synthesis becomes apparent. It is to be hoped that it will be energetically attacked during the next decade.

By the action of emulsin on a concentrated aqueous solution of galactose Bourquelot and Aubry have obtained two galactobioses. The one form was obtained in little spherical masses with a taste slightly sweeter than that of lactose. It had $\alpha_D + 53°$ and showed mutarotation. The behaviour towards emulsin is not stated. The second modification crystallises in needles $\alpha + 35°$ showing mutarotation. It is hydrolysed by emulsin. The behaviour of this second isomeride is not unlike the galactosidogalactose synthesised by Fischer and Armstrong from β-acetochlorogalactose.

The formation of two isomerides in this manner is of the greatest interest and the further study of their relationship is of much importance.

In the case of invertase the evidence is most definite that the enzyme from yeast accomplishes a complete hydrolysis of sucrose to glucose and fructose and that no synthesis takes place. This reaction does not establish an equilibrium and is not a reversible or balanced change. This problem was investigated with the greatest care by Armstrong in 1901 and by Hudson in 1914 with all the refinement which the modern methods of experiment permitted.

It is well known from the work of Pavy and Bywaters that living yeast forms glycogen when brought into excess of sugar solution. The enzymes involved in this synthesis are probably to some extent still present and active in yeast juice, as Cremer found that in yeast juice, free from glycogen, in presence of sugar, a substance was slowly formed which gave the characteristic glycogen reactions. Harden and Young find that one or more dextro-rotatory polysaccharides are produced during alcoholic fermentation by non-living yeast preparations. It is not settled whether these polysaccharides are formed from the glucose and fructose themselves or as the result of enzyme action on the products of hydrolysis of the hexose phosphate formed from them.

CHAPTER VII.

THE NATURAL GLUCOSIDES.

THE term glucoside is applied to a large number of bodies having the property in common of furnishing a '*glucose*' and one or more other products when hydrolysed by acids. They are resolved with the addition of the elements of water into simpler compounds. Representatives of nearly every class of organic compound occur in plants, chiefly in the fruit, bark and roots, in combination with a sugar which is in most cases dextroglucose. These compounds are glucose ethers of alcohols, acids, phenols, etc. ; they correspond in structure to the simple methyl glucosides, and the general formula of a glucoside is accordingly written :—

$$CH_2(OH).CH(OH).CH.[CH.OH]_2.CH-O-R$$
$$\qquad\qquad\qquad\qquad\quad \underbrace{\qquad\qquad O \qquad\qquad}$$

where R represents the organic radicle. It is noteworthy that the vegetable bases are only seldom found in the form of glucosides.

The glucosides correspond to a certain extent to the paired glucuronic acid derivatives previously mentioned. In both instances more or less reactive specific substances are combined with the sugar residue to form indifferent and frequently more soluble substances.

Glucosides are obtained by extraction of the plant substance with water or alcohol, an operation conveniently performed in a Soxhlet apparatus. It is necessary in the majority of cases first to destroy the accompanying enzyme when water is used as solvent. If this operation be omitted the glucoside is destroyed in the process of extraction. The purification of the extract is often a matter of difficulty owing to the scanty proportion of glucoside present.

The glucosides as a class are generally colourless crystalline solids, having a bitter taste and lævo-rotatory optical power. Some of the best-known glucosides are the amygdalin of the almond and other rosaceous plants, the salicin of the willow and the sinigrin of the cruciferæ.

The glucosides are all hydrolysed by heating with mineral acids to sugar and an organic residue. They are decomposed at very different

rates, some glucosides (e.g. gynocardin) being extremely resistant to acid hydrolysis.

In the majority of cases the glucosides are hydrolysed by enzymes. The appropriate enzyme is contained in the same plant tissue, but in different cells, gaining access to the glucoside only when the tissue is destroyed. A great number of such enzymes exist, but it is too much to say that each glucoside has a special enzyme for its decomposition. The best-known glucoside-splitting enzymes are the emulsin of almonds and the myrosin of black mustard seeds. Both these enzymes can effect hydrolysis of a number of glucosides.

Emulsin is especially wide in its action. Since it is the specific enzyme for β-alkyl glucosides, all glucosides hydrolysed by it are regarded as derivatives of β-glucose, though the fact that emulsin is a mixture of enzymes must not be lost sight of. No glucoside derivative of α-glucose has so far been isolated from plants.

The hydrolysis of glucosides by myrosin is undoubtedly connected with their sulphur content.

The majority of the glucosides are derived from dextro-glucose, but since more attention has been paid to the group, glucosides derived from a number of other carbohydrates have been discovered in plants, and there is little doubt that fresh investigation will extend their number. Glucosides are known which are derived from *d*- and *l*-arabinose, *d*-xylose, *d*-ribose, from rhamnose and other methyl pentoses, and from galactose, mannose and fructose. Glucosides containing carbohydrates other than glucose require special enzymes to effect their hydrolysis.

Galactose has been identified in convallamarin, digitonin, robinin, sapotoxin, solanin. Mannose is found only in strophantin.

Fructose is found in alliin (from garlic), and in the saponins from *Sapindus rarak* and *Aesculus hippocastanum.*

Rhamnose is a constituent of baptisin, convallamarin, datiscin, frangulin, fustin, glycyphyllin, hesperidin, campferitrin, ouabain, naringin, quercitrin, robinin, rutin, *Sapindus*-saponin, solanin, strophantin, trifolin, turpethein, xanthorhamnin.

Pentoses or methylpentoses have also been found in antiarin, barbaloin, convolvulin, gentiin, jesterin, quinovin, saponin, turpethein, vernin, vicianin.

Some glucosides yield two or more monosaccharides on hydrolysis. In such cases these are united as di- or trisaccharides. Using appropriate enzymes, the sugar groups may be removed one at a time, and new glucosides are formed. Thus amygdalin contains two glucose

residues, one of which is removed by an enzyme present in yeast and termed amygdalase. The new glucoside so formed was termed mandelonitrile glucoside: it has since been found in plants and named prunasin.

Both on account of the very small quantity of a glucoside usually present in a plant, and the fact that glucosides do not as a rule form insoluble characteristic derivatives which allow of their isolation, it is difficult to discover new glucosides and still more so to determine their nature. The introduction of biochemical methods has much facilitated work of this kind. Bourquelot's biological method has led to the discovery of several new glucosides, and ter Meulen has established the nature of the sugar component in several instances. Ter Meulen makes use of the fact (p. 122) that an enzyme is only compatible with and therefore only enters into combination with that sugar, the simple glucosidic compounds of which it is able to hydrolyse. He has investigated the rate of hydrolysis of a glucoside by the appropriate enzyme in presence of a number of the simple sugars. Only one of these sugars retards the change; the others are almost without influence. The glucoside in question is considered to be a derivative of that sugar which retarded the hydrolysis.

For instance, rhamninose alone retards the hydrolysis of xanthorhamnin; glucose alone retards the decomposition of salicin or of amygdalin. In the case of glucosides of which the nature of the sugar component was not absolutely established, it was shown that aesculin, arbutin, coniferin, indican, sinigrin and several other glucosides containing mustard oils are derivatives of *d*-glucose.

Bourquelot's biological method of examining plants for glucosides consists in the addition of emulsin to an extract of the plant and the determination of the changes in optical rotation and cupric reducing power after a period of incubation. A change indicates the presence of β-glucosides and its magnitude gives a rough indication of their quantity.

In this manner taxicatin, $C_{13}H_{22}O_7$, has been discovered in *Taxus baccata* (Lefebvre) and the presence of aucubin demonstrated in a number of species of plantago (Bourdier).

The use of invertase in the same manner affords a test for the presence of sucrose or raffinose.

A number of the better-known glucosides are given in the following table which also shows the products of hydrolysis. They are classified under alcohols, phenols, aldehydes, etc., according to the nature of the non-sugar part of the molecule.

TABLE XIX.

Natural Glucosides.

Glucoside.	M.p.	Products of Hydrolysis.
		Phenols.
Arbutin . . . $C_{12}H_{16}O_7$	200°	Glucose + hydroquinone
Baptisin . . . $C_{26}H_{32}O_{14}$	240°	Rhamnose + baptigenin
Glycyphyllin . . $C_{21}H_{24}O_9$	175°	Rhamnose + phloretin
Hesperidin . . $C_{50}H_{60}O_{27}$	251°	Rhamnose + 2 glucose + hesperetin
Iridin . . . $C_{24}H_{26}O_{13}$	208°	Glucose + irigenin
Methyl arbutin . $C_{13}H_{18}O_7$	175°	Glucose + hydroquinone methyl ether
Naringin . .	170°	Rhamnose + glucose + narigenin
Phloridzin . . $C_{21}H_{24}O_{10}$	170°	Glucose + phloretin
		Alcohols.
Coniferin . . $C_{16}H_{22}O_8$	185°	Glucose + coniferyl alcohol
Populin . . . $C_{20}H_{22}O_8$	180°	Glucose + saligenin + benzoic acid
Salicin . . . $C_{13}H_{18}O_7$	201°	Glucose + saligenin
Syringin . . $C_{17}H_{24}O_9$	191°	Glucose + syringenin
		Aldehydes.
Amygdalin . . $C_{20}H_{27}O_{11}N$	200°	2 Glucose + *d*-mandelonitrile
Dhurrin . . . $C_{14}H_{17}O_7N$	—	Glucose + *p*-oxymandelonitrile
Helicin . . . $C_{13}H_{16}O_7$	—	Glucose + salicylaldehyde
Linamarin . . $C_{10}H_{17}O_6N$	141°	Glucose + acetonecyanhydrin
Prulaurasin . . $C_{14}H_{17}O_6N$	122°	Glucose + racemic mandelonitrile
Prunasin . . $C_{14}H_{17}O_6N$	147°	Glucose + *d*-mandelonitrile
Salinigrin . . $C_{13}H_{16}O_7$	195°	Glucose + *m*-oxybenzaldehyde
Sambunigrin . . $C_{14}H_{17}O_6N$	151°	Glucose + *l*-mandelonitrile
Vicianin . . . $C_{19}H_{25}O_{10}N$	160°	Glucose + arabinose + *d*-mandelonitrile
		Acids.
Convolvulin . . $C_{54}H_{96}O_{27}$	150°	Glucose + rhodeose + convolvulinolic acid
Gaultherin . . $C_{14}H_{18}O_8$	100°	Glucose + methylsalicylate
Jalapin . . . $C_{44}H_{56}O_{16}$	131°	Glucose + jalapinolic acid
		Oxycumarin Derivatives.
Aesculin . . $C_{15}H_{16}O_9$	205°	Glucose + aesculetin
Daphnin . . $C_{15}H_{16}O_9$	200°	Glucose + daphnetin
Fraxin . . . $C_{16}H_{18}O_{10}$	320°	Glucose + fraxetin
Scopolin . . $C_{22}H_{28}O_{14}$	218°	2 Glucose + scopoletin
Skimmin . . $C_{15}H_{16}O_8$	210°	Glucose + skimmetin
		Oxyanthraquinone Derivatives.
Frangulin . . $C_{21}H_{20}O_9$	228°	Rhamnose + emodin
Polygonin . . $C_{21}H_{20}O_{10}$	202°	Glucose + emodin
Ruberythric acid . $C_{26}H_{28}O_{14}$	258°	Glucose + alizarin
Rubiadin . . $C_{21}H_{20}O_9$	—	Glucose + methylxanthofurfurin
		Oxyflavone Derivatives.
Apiin . . . $C_{26}H_{28}O_{14}$	228°	Apiose + apigenin
Campferitrin (Robinin) . . $C_{33}H_{40}O_{19}$	201°	Glucose + 2 rhamnose + campferol
Euxanthic acid (magnesium salt) $C_{19}H_{16}O_{10}$	—	Glucuronic acid + euxanthone
Fustin . . . $C_{36}H_{26}O_{14}$	218°	Rhamnose + fisetin
Gossypitrin . . $C_{21}H_{20}O_{13}$	—	Glucose + gossypetin
Incarnatrin . . $C_{21}H_{20}O_{12}$	242°	Glucose + quercetin
Isoquercitrin . . $C_{21}H_{20}O_{12}$	217°	Glucose + quercetin
Lotusin . . . $C_{28}H_{31}O_{16}N$	—	2 Glucose + HCN + lotoflavin
Quercimeritrin . $C_{21}H_{20}O_{12}$	247°	Glucose + quercetin

TABLE XIX. (*continued*).

Glucoside.	M.p.	Products of Hydrolysis.
Quercitrin . . $C_{21}H_{20}O_{11}$	183°	Rhamnose + quercetin
Rutin . . . $C_{27}H_{30}O_{16}$	184°	Glucose + rhamnose + quercetin
Serotin . . . $C_{21}H_{20}O_{12}$	245°	Glucose + quercetin
Sophorin . . $C_{27}H_{30}O_{16}$	—	Rhamnose + glucose + sophoretin
Thujin . . . $C_{21}H_{20}O_{11}$	183°	Quercetin + traces of another glucoside
Xanthorhamnin . $C_{34}H_{42}O_{20}$	—	2 Rhamnose + galactose + rhamnetin
		Mustard Oils.
Glucocheirolin $C_{11}H_{20}O_{11}NS_3K$, H_2O	160°	Glucose + cheirolin
Glucotropaolin . $C_{14}H_{15}O_9NS_2K$	—	Glucose + benzyl isothiocyanate + $KHSO_4$
Sinalbin . . $C_{30}H_{42}O_{15}N_2S_2$	138°	Glucose + sinapin acid sulphate + acrinyl-isothiocyanate
Sinigrin . . $C_{10}H_{16}O_9NS_2K$	126°	Glucose + allyl isothiocyanate + $KHSO_4$
		Anthocyans.
Cyanin . . . $C_{27}H_{30}O_{16}$	203°	2 Glucose + cyanidin
Delphinin . . $C_{41}H_{38}O_{21}$	200°	2 Glucose + 2 *p*-oxybenzoic acid + delphinidin
Idain . . . $C_{21}H_{20}O_{10}$	—	Galactose + cyanidin
Malvin . . . $C_{29}H_{34}O_{17}$	165°	2 Glucose + malvidin
Myrtillin . . $C_{22}H_{22}O_{12}$	—	Glucose + myrtillidin
Oenin . . . $C_{23}H_{24}O_{12}$	—	Glucose + oenidin
Pelargonin . . $C_{27}H_{30}O_{15}$	180°	2 Glucose + pelargonidin
		Digitalis Group.
Cymarin . . . $C_{30}H_{44}O_9$	138°	Cymarose (digitoxose methyl ether) + strophanthidin
Digitalin . . $C_{35}H_{56}O_{14}$	217°	Glucose + digitalose + digitaligenin
Digitonin . . $C_{54}H_{92}O_{28}$	225°	Glucose + galactose + digitogenin
Digitoxin . . $C_{34}H_{54}O_{11}$	145°	2 digitoxose + digitoxigenin
Gitalin . . . $C_{28}H_{48}O_{10}$	155°	Digitoxose + anhydrogitaligenin
Gitin . . . $C_{54}H_{92}O_{28}$	265°	2 Galactose + digitogenin
Gitonin . . . $C_{49}H_{80}O_{23}$	272°	3 Galactose + pentose + gitogenin
Strophanthin . $C_{36}H_{54}O_{15}$	—	Strophantibiose methyl ether + strophanthidin
		Sapogenins.
Agrostemma sapotoxin $(C_{17}H_{26}O_{10})_2$	—	4 Sugars + $C_{10}H_{16}O_2$
Caulophyllosaponin $C_{66}H_{104}O_{17}$	—	
Caulosaponin . $C_{54}H_{88}O_{17}$	—	
Digitonin . . $C_{54}H_{92}O_{28}$	—	2 Glucose + galactose + digitogenin
Digitosaponin . $C_{54}H_{92}O_{28}$	—	Pentose + digitosapogenin
Gypsophila (Levant sapotoxin) . $(C_{17}H_{26}O_{10},H_2O)_2$	—	Glucose + galactose + $C_{10}H_{16}O_2$
α-Hederin . . $C_{42}H_{66}O_{11}$	—	Arabinose + rhamnose + α-hederogenin
Jegosaponin . . $C_{55}H_{80}O_{25}$	—	Glucose + glucuronic and tiglic acids + 2 sapogenins
Parillin . . . $C_{26}H_{44}O_{10}$	—	2 Sugars + parigenin, $C_{28}H_{46}O_4$
Phytosterolins . $C_{33}H_{56}O_6$	—	Glucose + a sitosterol
Polysciasaponins . $C_{22}H_{36}O_{10}$ and $C_{25}H_{42}O_{10}$	—	
Quillaic acid . . $C_{19}H_{30}O_{10}$	—	
Quillaja sapotoxin . $C_{17}H_{26}O_{10}$	—	
Saporubrin . . $(C_{18}H_{28}O_{10})_4$	—	Several sugars + $C_{14}H_{22}O_2$
Sarsasaponin . $C_{44}H_{76}O_{20}$, $7H_2O$	—	3 Glucose + sarsasapogenin, $C_{26}H_{41}O_2(OH)$
Smilacin . .	—	

TABLE XIX. (*continued*).

Glucoside.	M.p.	Products of Hydrolysis.
		Various.
Aucubin . . . $C_{13}H_{19}O_8$	—	Glucose + aucubigenin
Barbaloin . . $C_{20}H_{18}O_9$	—	*d*-arabinose + aloemodin
Calmatambin . . $C_{19}H_{38}O_{13}$	144°	Glucose + calmatambetin
Datiscein . . $C_{21}H_{24}O_{11}$	190°	Rhamnose + datiscetin
Dibenzoylglucoxylose $C_{25}H_{28}O_{12}$, H_2O	148°	Glucoxylose + benzoic acid
Gentiin . . . $C_{25}H_{28}O_{14}$	274°	Glucose + xylose + gentienin
Gentiopicrin . . $C_{16}H_{20}O_9$	191°	Glucose + gentiogenin
Gynocardin . . $C_{13}H_{19}O_9N$	162°	Glucose + HCN + $C_6H_8O_4$
Indican . . . $C_{14}H_{17}O_6N$	100°	Glucose + indoxyl
Quinovin . . $C_{30}H_{48}O_8$	—	Quinovose + quinovaic acid
Saponarin . . $C_{15}H_{14}O_7$	—	Glucose + saponaretin
Vernin . . . $C_{10}H_{13}O_5N_5$	—	*d*-Ribose + guanine

The better-known glucoside-splitting enzymes are grouped in Table XX. together with the glucosides they decompose. Emulsin from almonds hydrolyses aesculin, amygdalin, androsin, arbutin, aucubin, bankankosin, calmatambin, conferin, daphnin, dhurrin, gentiopicrin, helicin, incarnatrin, indican, melatin, oleuropein, picein, prulaurasin, prunasin, salicin, sambunigrin, syringin, taxicatin, verbenalin, etc.

TABLE XX.

GLUCOSIDOCLASTIC ENZYMES.

Enzyme.	Hydrolyses.
Emulsin	Many natural glucosides Synthetical β-glucosides
Prunase	Prunasin and many other natural glucosides
Amygdalase	Amygdalin
Gaultherase	Gaultherin
Linase	Linamarin
Myrosin	Sinigrin and sulphur glucosides
Rhamnase	Xanthorhamnin

The Principal Glucosides.

A few only of the glucosides have been selected for detailed comment, more particularly for the purpose of showing the relationship between their structure and their distribution in plants. Such data, when more complete, will afford preliminary material for the differentiation of species upon a purely chemical basis, as has been indicated by Miss Wheldale. At present, since the knowledge of the glucosides is chiefly based on the investigation of substances used for medicinal purposes, only a beginning has been made in this direction.

Phenolglucosides.

Arbutin, a colourless, bitter, crystalline substance, is obtained, together with methylarbutin, from the leaves of the bear berry, a small evergreen shrub (*Arbutus uva ursi*), and from many genera in the *Ericaceæ*, and yields hydroquinone and glucose when hydrolysed by means of emulsin or mineral acids :—

$$C_{12}H_{16}O_7 + H_2O = C_6H_{12}O_6 + C_6H_6O_2$$

Hydroquinone is a powerful antiseptic: hence the pharmacological value of arbutin, which has also a diuretic action. Methyl arbutin was one of the first glucosides to be artificially synthesised. Michael prepared it by the interaction of hydroquinone methyl ether and acetochloro glucose.

Commercial arbutin contains methyl arbutin; to purify it, it is dissolved in alcohol, precipitated by potassium hydroxide and the precipitate collected, washed and decomposed with calcium carbonate (Hérissey).

Mannich states that a better, but still imperfect, method of separation of pure arbutin from the mixture is to take advantage of the additive compound formed by arbutin and hexamethylene tetramine.

When arbutin is hydrolysed by emulsin the quinol formed becomes slightly oxidised by the oxydase present in the enzyme and the solution darkens in colour. Methyl arbutin, which yields quinol methyl ether on hydrolysis, does not darken in solution. It is hydrolysed more rapidly than arbutin.

Bourquelot and Fichtenholz have made an extensive study of the distribution of arbutin in the leaves of Pyrus species. Pear leaves (*Pyrus communis*) contain as much as 1·2 to 1·4 per cent. of the glucoside, which can be extracted by ethyl acetate. None could be detected in *Cydonia vulgaris*, *Malus communis*, *Sorbus aucuparia*, or *S. torminalis*, all of which were at one time classed with *Pyrus*: the modern classification is thus justified on biochemical grounds.

The leaves of certain varieties of Pyrus turn black when they fall; these contain arbutin which is hydrolysed to quinol by the leaf enzyme, the quinol in turn being acted on by an oxydase to form the black substance. In other varieties a golden yellow tint first appears which then gives place to black. These varieties are shown to contain methyl arbutin; they produce at first a yellow and not a black oxidation product.

Phloridzin, which is found in the bark of apple, pear, cherry, plum and other rosaceous trees, is remarkable for the property it possesses of causing glucosuria when taken internally. Emulsin is without

action on it: mineral acids form glucose and phloretin, $C_{15}H_{14}O_5$, which is a condensation product of *p*-oxyhydratropic acid and phloroglucinol.

Phloridzin has the formula—

$$(C_6H_{11}O_5 \cdot O)(OH)_2C_6H_2 \cdot CO \cdot CHMe \cdot C_6H_4(OH)$$

When phloridzin is treated with barium hydroxide it is hydrolysed to phloretic acid and phlorin (phloroglucinol glucoside):—

$$C_6H_3(OH)_2 - O - C_6H_{11}O_5,$$

which last is identical with the phloroglucinol glucoside synthesised by Fischer and Strauss. Phloretin is a component also of *Glycyphyllin*, the glucoside of the leaves of *Smilax glycyphylla*, where it is combined with rhamnose.

The phloroglucinol complex is present in the aromatic part of a large number of glucosides.

Salicin, a colourless, crystalline, bitter substance, is the active constituent of willow bark; it has long been used as a remedy against fever and in cases of acute rheumatism. It is hydrolysed by emulsin to glucose and saligenin (*o*-oxybenzyl alcohol), and has the formula $C_6H_{11}O_5 \cdot O \cdot C_6H_4 \cdot CH_2OH$. Saligenin yields salicylic acid on oxidation, but has the advantage of being less irritant than this acid or its salts, and therefore does not produce digestive disturbances when administered medicinally.

Salicin occurs in many but not all species of *Salix*, also in poplars and in the flower buds of meadow-sweet, *Spiræa ulmaria*. In the willow it is found in the leaves and female flowers as well as in the bark; the leaves and twigs of willows also contain a specific enzyme, salicase, which hydrolyses it (Sigmund).

Salicin forms bromo and chloro derivatives which are hydrolysed by emulsin.

When shaken with benzoyl chloride a monobenzoyl derivative is obtained in which the benzoyl group is in the sugar nucleus and not attached to the alcohol group of saligenin. This compound is identical with the natural glucoside *populin* found in the bark of a number of species of poplar (*Populus*). According to Weevers populin is hydrolysed by an enzyme in *Populus monilifera* to salicin and benzoic acid. Emulsin is without action on populin.

Helicin, the glucoside of salicylic aldehyde, is obtained on oxidation of salicin with dilute nitric acid. It has not been found to occur naturally, but was synthesised by Michael from salicylaldehyde and acetochloro glucose. Emulsin hydrolyses helicin and also its hydrazone and oxime. Helicin was coupled by Fischer with hydrogen cyanide to yield a synthetic cyano-genetic glucoside from which a further series of glucosides were obtained.

Salinigrin, the glucoside of *m*-hydroxy benzaldehyde, is isomeric with helicin. It was only found in one species (*Salix discolor*) out of thirty-three samples of willow and poplar examined by Jowett and Potter.

Gaultherin, the glucoside of methyl salicylate, is widely distributed in plants. It is not hydrolysed by emulsin, but gaultherase, the enzyme of *Gaultheria procumbens* and other plants, and mineral acids decompose it into glucose and methyl salicylate.

Coniferin, the glucoside of the fir-tree, is of importance as the starting-point for the synthesis of vanillin which is formed from it by oxidation with chromic acid.

It yields glucose and coniferyl alcohol when hydrolysed by emulsin, and has the formula :—

$$(C_6H_{11}O_5 . O) . C_6H_3(OMe) . CH : CH . CH_2OH$$

By careful oxidation glucovanillin is formed, and this may be oxidised to glucovanillic acid or reduced to glucovanillyl alcohol. All three glucosides are hydrolysed by emulsin.

A methoxy coniferin is *syringin*, the glucoside of the Syringa, which is likewise hydrolysed by emulsin to syringenin (methoxy coniferyl alcohol).

Coumarin Glucosides.—Coumarin is very widely distributed in plants: there can be little doubt that it is present in the form of a glucoside but this has not yet been isolated. Several glucosides containing hydroxycoumarins are known.

Skimmin, $C_{15}H_{16}O_8$, a constituent of *Skimmia japonica*, is the glucoside of 4-hydroxycoumarin (skimmetin), which is isomeric if not identical with umbelliferone.

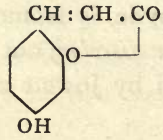
Aesculin, $C_{15}H_{16}O_9$, found in horse-chestnut bark (*Aesculus hippocastanum*) and *Daphnin*, a constituent of several species of *Daphne*, are glucosides of isomeric dihydroxy coumarins named æsculetin and daphnetin respectively.

Scopolin, present in *Scopolia japonica*, is æsculin monomethyl ether. It is said to contain two molecules of glucose.

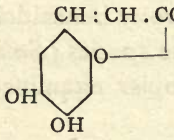
Limettin, the dimethyl ether of æsculin, is found in citrus.

Fraxin, $C_{16}H_{18}O_{10}$, found in the ash, in species of *Aesculus* and in *Dienvilla*, is the glucoside of a monomethyl ether of trihydroxycoumarin termed fraxetin. The position of the methyl group is uncertain.

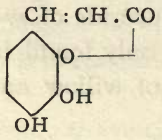
The following formulæ show the relation of these glucosides: it is not known which hydroxyl is attached to the glucose residue :—



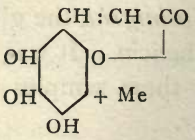
Skimmetin.



Aesculetin.



Daphnetin.



Fraxetin.

Madder.

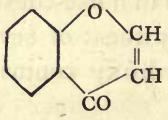
Madder, the ground root of *Rubia tinctorum*, which was long considered as the most important dye-stuff and has been cultivated from remote antiquity, consists of a number of glucosides of which the most important is ruberythric acid. This is composed of two molecules of glucose and alizarin, i.e. dihydroxyanthraquinone. The glucose molecules are probably united as a disaccharide since the glucoside is strongly acid and forms red coloured salts indicating the presence of a free hydroxyl group. Since its synthesis by Graebe and Liebermann alizarin has been manufactured entirely from anthraquinone and the madder industry destroyed.

The madder contains an enzyme, erythrozym, which hydrolyses the glucoside.

Other glucosides present in madder are those of *purpurin* which is trihydroxyanthraquinone: *xanthopurpurin* which is a dihydroxyanthraquinone isomeric with alizarin and *rubiadin*, which is a methyl derivative of xanthopurpurin united to one molecule of glucose.

Hydroxyflavone or Anthoxanthinglucosides.

The great majority of the soluble yellow plant pigments are glucosides derived from flavone or xanthone; that is, they contain the benzopyrone nucleus :—



In the pigments so far studied this nucleus contains one or more hydroxyl groups and is united to the simple aromatic compounds benzene, phenol, catechol, resorcinol or pyrogallol. The structure of the pigments has been deduced by the study of their decomposition products and in many instances confirmed by their synthesis: much of the progress in this group is due to the researches of A. G. Perkin. Syntheses of many of the hydroxyflavones have been accomplished by a method due to Kostanecki.

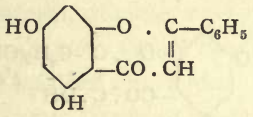
The sugar residue may be either glucose or rhamnose, or some other monosaccharide or even a disaccharide, the methylpentoses being

common in this group. In addition to the differences in the non-sugar part of the molecule there are several isomerides possible according to which phenolic group is concerned in the attachment to the sugar. Such differences in constitution will correspond with differences in the properties of the several glucosides, especially in their behaviour towards acids.

The flavone glucosides are in general colourless or nearly so and it would appear probable that when a yellow tint is due to their agency it is to be traced, as in the case of the yellow cotton flower, to their presence as a potassium or other salt. The sugar-free pigments, which occur also in the free state along with the glucosides, are yellow crystalline solids giving a number of characteristic colour reactions.

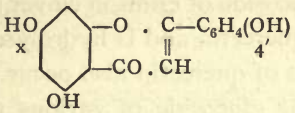
The following hydroxyflavones or their glucosides have so far been isolated from plants :—

The glucoside of chrysin (1 : 3-dihydroxyflavone)



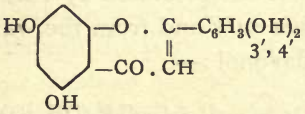
has not been itself isolated, but the flavone occurs in various species of poplar and mallows.

Apiin, the glucoside present in the leaves of parsley, celery, etc., is hydrolysed to glucose, apiose (a sugar of abnormal structure with five carbon atoms) and apigenin (1 : 3 : 4′-trihydroxy flavone) :—

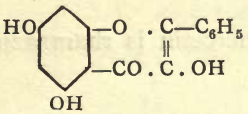


According to Perkin the sugar residue is united to the hydroxyl group marked x. Apigenin has been identified by Wheldale as the basis of the ivory white of antirrhinum flowers.

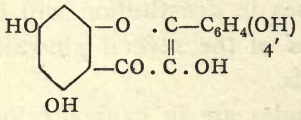
The glucoside of luteolin (1 : 3 : 3′ : 4′ : tetrahydroxy flavone), the colouring matter of *Reseda luteola* and *Genista tinctoria*, has likewise not been isolated :—



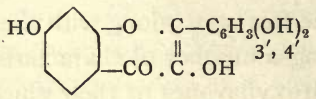
The glucoside of galangin (1 : 3-dihydroxy flavonol) occurs in the root of *Galanga* :—



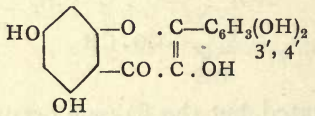
Campferitrin (Robinin) is the glucoside of the white azalea (*Robinia pseudacacia*) and of Java indigo. It is composed of glucose, rhamnose (2 molecules) and campherol (1 : 3 : 4′-trihydroxy flavonol) :—



Fustin, the glucoside of fustic (*Rhus cotinus*) and perhaps aiso of *Quebracho colorado*, is hydrolysed to rhamnose and 2 molecules of fisetin 3 : 3′ : 4′-trihydroxyflavonol) :—



Quercitrin is the glucoside of the oak bark. It is easily hydrolysed by acids to rhamnose and quercetin (quercitin) – 1 : 3 : 3′ : 4′-tetra-hydroxyflavonol :—



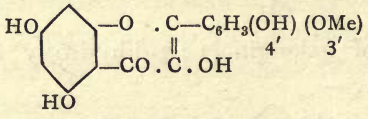
Quercetin is very widely distributed in plants from many of which the glucoside has not been isolated. It frequently occurs, together with other pigments of the group. It follows indigo and alizarin in industrial importance as a natural dye-stuff.

Incarnatrin, the glucoside of crimson clover (*Trifolium incarnatum*), contains glucose and quercetin and is hydrolysed by emulsin.

Monomethyl ethers of quercetin also occur as glucosides.

Xanthorhamnin, the glucoside of various species of rhamnose, is composed of galactose, rhamnose (two molecules) and rhamnetin (monomethyl quercetin). According to Tanret the methoxyl group occupies positions 1 or 3. Wheldale considers it replaces the OH group in the γ-pyrone ring. Frangulin, from *Rhamnus frangula*, is not identical with xanthorhamnin, being a glucoside of emodin.

An iso-rhamnetin has been isolated by Perkin from an Indian dye, asbarg, from *Delphinium zalil* and from the wallflower : it is 1 : 3 : 4′-hydroxy 3′-methoxy flavonol :—



A dimethyl ether of quercetin is rhamnazin, present in the fruits of *Rhamnus* species.

When quercitrin is methylated with diazomethane (Herzig) the free hydroxyl groups are readily methylated and ultimately a penta-methyl quercitrin obtained, one methyl entering into the rhamnose molecule. On hydrolysis a tetramethyl quercetin is formed in which the hydroxyl in the pyrone ring is left unmethylated. The attachment of rhamnose and quercetin must accordingly take place through this hydroxyl.

Quercimeritrin obtained from the flowers of *Gossypium herbaceum* is composed of glucose and quercetin. Acids hydrolyse it with difficulty. The sugar residue is supposed to be attached either to the hydroxyl group 1 or 3.

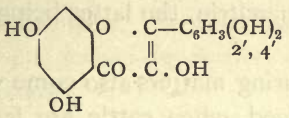
Iso-quercitrin accompanies quercimeritrin in cotton flowers. It differs from it in being easily hydrolysed by acids to glucose and quercetin.

Rutin, which is widely distributed in plants, is hydrolysed with difficulty by acids to quercetin, glucose, and rhamnose.

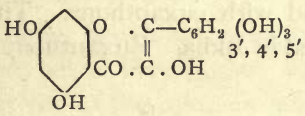
Thujin, in *Thuja occidentalis* (arborvitae), consists of quercitrin accompanied by traces of another glucoside.

Serotin, present in *Prunus Serotina*, is easily hydrolysed by acids to glucose and quercetin.

The glucoside of yellow wood (*Morus tinctoria*) contains morin (1 : 3 : 2′ : 4′-tetrahydroxyflavonol) :—

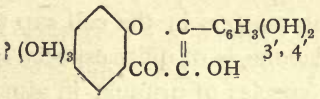


Myricetin (1 : 3 : 3′ : 4′ : 5′-pentahydroxyflavonol) :—



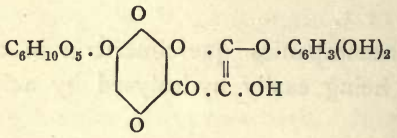
is found in the leaves of *Rhus* species and in the bark of *Myrica magi.*

Gossypitrin, one of the glucosides present in Egyptian cotton flowers, yields on hydrolysis glucose and gossypetin, $—C_{15}H_{10}O_8$, it has the following formula though the position of the hydroxyl groups in the tetrahydroxybenzene nucleus has not yet been determined with certainty. The sugar is attached to the tetrahydroxybenzene nucleus :—



Quercetagetin, from the flowers of the African marigold (*Tagetes patula*), is very closely allied to gossypetin, both containing tetrahydroxybenzene and catechol nuclei. It perhaps has the structure of a 1 : 2 : 4 : 3′ : 4′-pentahydroxyflavonol, though the 1 : 2 : 3 : 3′ : 4′ alternative is also possible.

Gossypetin forms an interesting quinone, gossypetone, on hydrolysis, which resembles the quercetone obtained from quercetin by the action of chromic acid. Gossypitrin yields a similar quinone on oxidation by means of benzoquinone. Perkin suggests this has the structure :—

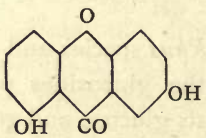


Perkin has compared the glucosides of a variety of cotton flowers. The red flowers of *G.-arboreum* contained isoquercitrin ; the yellow flowers of the Indian *G.-neglectum* contained gossypetrin and isoquercitrin ; whereas the yellow flowered Egyptian variety contained quercimeritrin as well. White flowered varieties *G.-Rossum* gave only very small quantities of a glucoside resembling apigenin, and the pink flowers of *G.-sanguineum* contained only traces of flavones.

The leaves and flowers of Upland cotton *G.-hirsutum* contain quercimeritrin and isoquercitrin, the latter being present in the petals only.

The xanthone colouring matters also come within this group.

Euxanthone is formed when cattle are fed with mango leaves. The urine contains euxanthic acid (Indian yellow) which is a combination of glucuronic acid with euxanthone. The pigment is made in Bengal and largely used in India. Euxanthone is 2 : 3′-dihydroxyxanthone :—



Gentisin, the yellow pigment present in *Gentiana lutea*, no doubt originally in the form of a glucoside, is 4-methoxy-2 : 3-dihydroxyxanthone.

The hydroxyflavone glucosides are so widely distributed in plants in the form of colouring matters in the cell sap that the occurrence of the parent substance, flavone, is of quite especial interest. It is present as the farina of many species of primula in almost pure condition, as

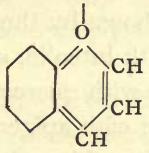
was shown by Hugo Müller. No opinion is expressed by him as to the physiological function which flavone exercises in the economy of the plant life, though the fact that it is excreted so freely would seem to imply that it is of no further use in the life process although its repellant action towards water is probably of importance. This observation has been confirmed by Shibata and Nagai, who find that the waxlike or powdery coverings of many plant organs contain flavone compounds secreted by the epidermis.

The same observers have examined the leaves, flowers, bark, wood, etc., of over 240 species of tropical plants and find that flavones were invariably present. They suggest that the flavone glucosides exert a protective action against the solar rays, especially those of short-wave length which are injurious to the living protoplasm, and evidence the fact that plants grown in the shade contain less flavone glucoside than those grown in the open. Similarly, plants provided with a heavy cuticle are usually poor in flavones. They further find that flavones and anthocyanins often interchange, showing they have the same protective function. Thus young shoots contain red anthocyanin which changes into the colourless flavone glucoside in the green organ and back into the anthocyanin before the fall of the leaves.

To detect the flavone the tissue is extracted with hot alcohol and a few cubic centimetres of the extract are heated with a drop of mercury, a little magnesium powder, and a few drops of concentrated hydrochloric acid in a test tube. In the presence of a flavone, reduction takes place with a vigorous generation of hydrogen gas and the production of a red colour.

Anthocyan Glucosides.

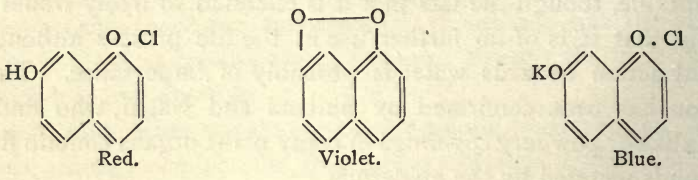
The soluble red, violet, and blue pigments of the cell sap are all glucosides. They are being investigated by Willstätter who has shown they are derivatives of the complex benzo-pyrilium nucleus :—



which differs from the benzopyrone nucleus of the hydroxyflavones in having a CH group n place of the CO group. The oxygen atom in the ring is strongly basic and forms quadrivalent stable salts with acids. The phenolic hydroxyl groups can form salts with alkali.

Willstätter explains the existence of the red, violet, and blue

colours as follows: the red is the acid salt, the blue is the potassium or metallic salt, and the violet is the anhydride of the pigment:—



Red. Violet. Blue.

The colour is due to the quinonoid structure of the molecule.

To prepare the anthocyans the material is extracted with cold glacial acetic acid and the extract precipitated with ether. A syrup is precipitated which is dissolved in warm aqueous picric acid solution: on cooling the crystalline picrate separates. It is converted into the chloride which can be crystallised from dilute alcohol containing hydrochloric-acid.

The anthocyans so far investigated are:—

(1) From cornflower and rose,
cyanin, $C_{27}H_{31}O_{16}Cl$ = 2 mols. glucose + cyanidin.

(2) From cranberry,
idain, $C_{21}H_{21}O_{10}Cl$ = galactose + cyanidin.

(3) From blue grapes,
œnin, $C_{23}H_{25}O_{12}Cl$ = glucose + œnidin.

(4) From whortleberry,
myrtillin, $C_{22}H_{23}O_{12}Cl$ = glucose + myrtillidin.

(5) From larkspur,
delphinin, $C_{41}H_{39}O_{21}Cl$ = 2 mols. glucose + 2 mols. *p*-oxy-benzoic acid + delphinidin.

(6) From geranium,
pelargonin, $C_{27}H_{3}O_{15}Cl$ = 2 mols. glucose + pelargonidin.

(7) From mallow,
malvin, $C_{29}H_{35}O_{17}Cl$ = 2 mols. glucose + malvidin.

(8) From pæony,
pæonin, $C_{28}H_{33}O_{16}Cl$ = 2 mols. glucose + pæonidin (= cyanidin methyl ether).

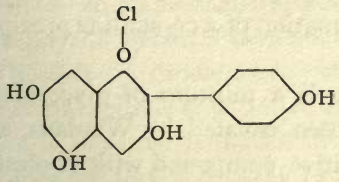
The close relationship which exists between the anthocyanidins and the anthoxanthins is shown by the fact that—

Cyanidin is isomeric with luteolin, campherol, and fisetin;

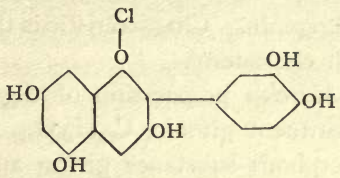
Delphinidin is isomeric with quercetin and morin;

Pelargonidin is isomeric with apigenin and galangin.

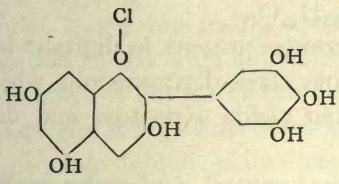
All three compounds give phloroglucinol when heated with alkali. Willstätter gives them the following constitutional formulæ:—



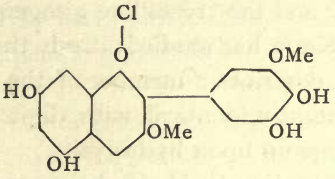
Pelargonidin.



Cyanidin.



Delphinidin.



Oenidin.

The interconversion of the anthoxanthins and the anthocyanidins is a matter of the greatest interest as it undoubtedly takes place in the plant probably under the influence of oxidative and reducing enzymes. Chemically the change is not easily effected though quercitin has been reduced to cyanidin by Everest. Pelargonidin has been synthesised by Willstätter.

The rôle of oxydases in the formation of the anthocyan pigments of plants has been studied by Keeble and Armstrong.

Digitalis Glucosides.

The leaves of the foxglove (*Digitalis purpurea*) contain apparently more than five glucosides which form the active constituents of digitalis, but their nature has been but scantily investigated.

Digitoxin, the most active principle, is insoluble in water; on hydrolysis it forms digitoxigenin and a sugar, $C_6H_{12}O_4$, digitoxose.

There are apparently two digitoxins, one of which forms a hydrate. The commercial product made by Merck changed from one form to the other in 1895. Digitoxose is very unstable and much of it is resinified when the glucoside is hydrolysed even with 0·5 per cent. hydrochloric acid.

Digitalin possesses in a high degree the physiological action of digitalis, decreasing the frequency and increasing the force of the beat of the heart; it yields digitaligenin, glucose, and digitalose, $C_7H_{14}O_5$, on hydrolysis.

Digitonin, which comprises one-half of the mixed glucosides of the seeds, belongs to the saponins: it dissolves sparingly in water, forming opalescent solutions which froth on agitation. It is hydrolysed to glucose (two molecules), galactose (two molecules) and

digitogenin. Characteristic is the formation of a crystalline precipitate with cholesterol.

Merck's preparation of digitonin is a mixture of glucosides; a constituent gitonin, $C_{49}H_{80}O_{23}$, has been isolated by Windaus, as an amorphous substance giving an additive compound with cholesterol. It is hydrolysed to galactose (three molecules), a pentose (one molecule) and the crystalline gitogenin, $C_{26}H_{42}O_4$.

Kraft has studied afresh the glucosides present in digitalis leaves and describes a member of the saponin class, digitosaponin, which is apparently identical with digitonin, but yields a pentose and digitosapogenin upon hydrolysis.

Gitalin, $C_{28}H_{48}O_{10}$, an amorphous, neutral and very sparingly soluble glucoside possessing physiological activity, was also isolated by Kraft. By evaporation of the alcoholic solution he obtained the crystalline anhydrogitalin, $C_{28}H_{46}O_9$. Both gitalin and its anhydro-derivative give the same products of hydrolysis, namely, digitoxose and anhydrogitaligenin, $C_{22}H_{34}O_5$.

Kiliani states, on the other hand, that gitalin itself is a mixture of glucosides, separable by fractional solution in water and a mixture of organic solvents into fractions differing in physiological action, solubility, and hydrolytic behaviour.

Some of the glucosides of digitalis seeds form crystalline addition products with amyl alcohol, thus allowing the separation of digitonin and gitonin in the precipitate from digitalin in the solution.

The work of Windaus and Hermanns has shown that cymarin, the active principle of Canadian hemp (*Apocynum cannabinum*), belongs to the digitalis group of glucosides. When hydrolysed it yields cymarigenin and a new sugar cymarose which behaves as the methyl ether of digitoxose. Cymarigenin is identical with apocyanamarin obtained by Moore from *Apocyanum androsæmifolium* and with strophanthidin from strophanthin-Kombe. An interesting relation between the three glucosides is thus established :—

$C_{34}H_{54}O_{11}$	=	$C_{22}H_{32}O_4$	+	$2C_6H_{12}O_4$
Digitoxin		Digitoxigenin		Digitoxose
$C_{30}H_{44}O_9$	=	$C_{23}H_{30}O_5$	+	$C_7H_{14}O_4$
Cymarin		Cymarigenin		Cymarose
		= Strophanthidin		= Digitoxose methyl ether
$C_{36}H_{54}O_{15}$	=	$C_{23}H_{30}O_5$	+	$C_{13}H_{24}O_{10}$
Strophanthin		Strophanthidin		Strophantobiose methyl ether.

Strophantobiose is said to be composed of mannose and rhamnose.

Hirohashi states that the youngest leaves of digitalis are the most active physiologically: they should be collected before inflorescence. There is no difference in activity between red and white flowers. Cultivated digitalis is as active as the wild variety and the first year's growth is as active as that of the second (Hatcher).

Oleander leaves contain two crystalline active glucosides similar to those in digitalis leaves. The whole of the active substances in oleander leaves are readily extracted by cold water: this solubility seems due to the large amount of a phenolic glucoside present in the leaves which is not a true tannin.

A careful study of the development of the glucosides in germinating and growing digitalis plants has been made by Straub. The amount of the glucosides was estimated by a pharmacological method, viz. by determining the number of lethal doses for a frog. The glucosides studied were digitalinum verum and digitalein, which are soluble in water, in the seeds and further digitoxin, which is insoluble in water but soluble in chloroform, and gitalin, soluble in both water and chloroform, in the leaves. The glucosides of the seeds are not reserve materials but disappear during germination and are stored in the leaves, in which organs they do not increase further in quantity.

The leaf glucosides are found in the earliest foliage leaves and continue to increase in quantity until they form one per cent. of the dried matter: it is supposed that they are only waste products of the metabolism of growth.

Indican.

Plants which yield indigo do not contain the colouring matter as such but in the form of a glucoside indican, which is readily extracted from the leaf by means of acetone. Indican yields glucose and indoxyl on hydrolysis; the indoxyl (colourless) undergoes further oxidation to indigotin (the blue colouring matter):—

$$\underset{\text{Indican.}}{C_{14}H_{17}O_6N} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Indoxyl.}}{C_8H_7ON} \qquad \underset{\text{Indoxyl.}}{2C_8H_7ON} + O_2 = 2H_2O + \underset{\text{Indigotin.}}{C_{16}H_{10}O_2N_2}$$

Indigotin is readily obtained on hydrolysing indican with dilute acids containing a little ferric chloride as an oxygen carrier, but the yield under these conditions is not quantitative. In the plant an oxydase plays an important part in the formation of indigotin.

Indican is also hydrolysed by a specific enzyme, indimulsin, which is present in the leaves of the indigo plant. Emulsin also slowly hydrolyses indican, but its action is far less intense than that of the *Indigofera* enzyme preparations. The yield of indigotin in this case is

also below the theoretical, especially when hydrolysis is slow: this is due to the great instability of indoxyl and in part also to the occlusion of indoxyl by the enzyme. It may be improved by adding a small quantity of sulphuric acid to the mixture at the commencement of the reaction. Technically it is of the greatest importance that the yield of natural indigo obtained on the manufacturing scale be a maximum.

Mustard Oil Glucosides.

A number of plants belonging to the cruciferæ yield glucosides containing sulphur. These give rise to mustard oils when hydrolysed by the enzyme myrosin which accompanies them in the plant. The best-known representatives of this class are sinigrin and sinalbin, found in the seeds of the black and white mustard. When the seed of black mustard is bruised and moistened, the odour of allylisothiocyanate is easily recognised. The myrosin and the glucoside are contained in separate cells in the seed, and do not interact until brought together by the solvent.

The recognition of an ethereal oil as the active principle of black mustard dates from 1730 (Boerhave). Bussy was the first to isolate the glucoside, which he termed potassium myronate, and the accompanying enzyme myrosin. Will and Körner gave the name sinigrin to the glucoside, and showed that it is hydrolysed to allylisothiocyanate, glucose, and potassium hydrogen sulphate:—

$$C_{10}H_{16}O_9NS_2K + H_2O = C_3H_5 . NCS + C_6H_{12}O_6 + KHSO_4$$

Sinigrin was subsequently investigated in detail by Gadamer, who proposed the formula—

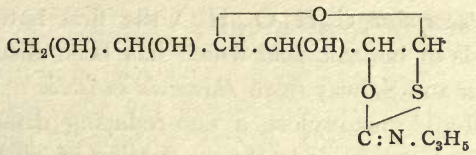
$$C_3H_5 . N : C(S . C_6H_{11}O_5) . O(SO_3K)$$

Schneider's isolation of the silver derivative of thioglucose from sinigrin tends to support this view.

When potassium methoxide is added to sinigrin, potassium sulphate separates at once, and on adding ammoniacal silver nitrate the silver salt of thioglucose is obtained, proving that the glucose molecule is attached to the sulphur atom in the glucoside:—

$$C_3H_5 . N : C . (OSO_3K) . S . C_6H_{11}O_5$$
$$\longrightarrow C_3H_5 . N : C(OMe) . S . C_6H_{11}O_5$$
$$\longrightarrow HS . C_6H_{11}O_5$$

At the same time another decomposition product, merosinigrin, is formed which is characterised by great stability: it forms a triacetate and may be a ring compound:—



It is not hydrolysed by emulsin or by yeast extract or any known enzyme other than myrosin. As hydrolysis proceeds, the increasing quantity of acid potassium sulphate formed renders the ferment less active and ultimately stops its action.

Guignard has very carefully investigated the localisation of myrosin in the plant. It occurs in special cells with finely granular contents which are free from starch, chlorophyll, fatty matter, and aleurone grains.

Sinalbin is likewise hydrolysed by myrosin, which accompanies it in the seeds, to glucose, sinalbin mustard oil (*p*-hydroxybenzylisothiocyanate) and acid sinapin sulphate :—

$$C_{30}H_{42}O_{15}N_2S_2 + H_2O = C_6H_{12}O_6 + C_7H_7O \cdot NCS + C_{16}H_{24}O_5N \cdot HSO_4$$

Barium hydroxide converts acid sinapin sulphate into choline and sinapinic acid :—

$$C_6H_2(OH)(OMe)_2 \cdot CH : CH \cdot CO_2H$$

It is of interest that the alcohol corresponding with this acid is syringenin, a constituent of the glucoside syringin.

Glucocheirolin, $C_{11}H_{20}O_{11}NS_3K$, H_2O, occurring in wallflower seeds, has been studied by Schneider and found to be a derivative of an aliphatic sulphone. Its probable constitution is—

$$CH_3 \cdot SO_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot N(O \cdot SO_3K) \cdot S \cdot C_6H_{11}O_5$$

It is hydrolysed to glucose and cheirolin by myrosin.

Pentosides.

Barbaloin, $C_{20}H_{18}O_9$, is hydrolysed to *d*-arabinose and aloemodin, $C_{15}H_{10}O_5$. This pentose was at first described under the name aloinose (Leger): it affords one of the rare instances of the natural occurrence of both *d* and *l* modifications of a carbohydrate (q.v. arabinose). l-*Arabinose* is a constituent of the saponins as well as of gums and pentosans.

Vernin, $C_{10}H_{13}O_5N_5,2H_2O$, is guanine-*d*-ribose. Originally discovered by Schulze in the seeds of *Lupinus luteus*, it was recognised as a pentoside by Schulze and Castoro. It is identical with the guanosin obtained by Levene and Jacobs from nucleic acid and with the pentoside obtained by Andrlik from molasses. The pentose was recognised as *d*-ribose by Levene and Jacobs and used by them for the synthesis of *d*-allose and *d*-altrose.

Dibenzoylglucoxylose, $C_{25}H_{28}O_{12},H_2O$, the first naturally occurring simple glucoside of benzoic acid which has been discovered, was obtained by Power and Salway from *Daviesia latifolia*. It is hydrolysed to benzoic acid and glucoxylose, a non-reducing disaccharide. Acid hydrolysis of the latter leads to the production of glucose and xylose.

Amygdalin.

Amygdalin is perhaps the best known and at the same time the most interesting of the glucosides; it has formed the subject of repeated and fruitful investigation ever since its discovery eighty-nine years ago, and even to-day the exact structure is not satisfactorily established. It is an example of a glucoside which contains nitrogen; on hydrolysis it yields benzaldehyde, hydrogen cyanide and two molecules of glucose. It is found in large quantities in bitter almonds and in the kernels of apricots, peaches, plums, and most fruits belonging to the Rosaceæ. It is the antecedent of the so-called essence of bitter almonds, and is widely used as a flavouring material. Like most glucosides it is a colourless, crystalline, bitter substance soluble in water.

The presence of hydrogen cyanide in the aqueous distillate of bitter almonds was observed at the very beginning of the nineteenth century by Bohm; the crystalline glucoside was first obtained by Robiquet and Boutron Charlard in 1830, who showed its connection with the essence of bitter almonds.

In 1837 Liebig and Wöhler found that amygdalin was hydrolysed by a certain nitrogenous substance, also existing in the almond, to which they gave the name emulsin, in accordance with the equation—

$$\underset{\text{Amygdalin.}}{C_{20}H_{27}O_{11}N} + 2H_2O = \underset{\text{Benzalde-hyde.}}{C_7H_6O} + \underset{\text{Hydrogen cyanide.}}{HCN} + 2\underset{\text{Glucose.}}{C_6H_{12}O_6}$$

They proved it to be a glucoside of benzaldehyde cyanhydrin.

Ludwig in 1856 pointed out that hot mineral acids hydrolyse amygdalin, giving rise to the same products as emulsin does. Schiff was the first to suggest that the two glucose molecules were united as a biose—

$$C_6H_5 . CH(CN) . O - C_6H_{10}O_4 . O . C_6H_{11}O_5,$$

and this view became generally accepted when it was shown by Fischer that amygdalin may be resolved by an enzyme, contained in yeast extract, into a molecule of glucose and one of a new glucoside which he termed mandelonitrile glucoside—

$$C_6H_5 . CH(CN) . O—C_6H_{11}O_5$$

Fischer came to the guarded conclusion that amygdalin was a derivative either of maltose or of a closely related diglucose. The view that amygdalin is a maltoside has passed into the literature (cf. Dunstan and Henry, British Association Report, York, 1906).

Recent work, however, does not support this supposition. Neither in its behaviour towards enzymes nor in its chemical properties does amygdalin behave as a maltoside.

When hydrolysed by means of strong hydrochloric acid, amygdalin gives *l*-mandelic acid, and Fischer's amygdonitrile glucoside is correspondingly *d*-mandelonitrile glucoside.[1]

Amygdalin at first sight seems to present an exception to the rule that enzymes which attack β-glucosides are strictly without action on α-glucosides, and vice versa. Emulsin hydrolyses amygdalin at both glucose junctions; an enzyme in yeast extract (maltase?) also attacks one of these. This junction must either be attackable by two distinct enzymes, or the enzymes in question must be mixtures and contain a common constituent. The latter hypothesis has proved to be correct.

Caldwell and Courtauld, in the course of a quantitative study of the hydrolysis of amygdalin by acids, showed that change takes place more readily at position Y in the molecule than at position X, as indicated in the formula—

$$C_6H_5 . CH(CN)\underset{X}{O} . C_6H_{10}O \ . \underset{Y}{O} . C_6H_{11}O_5$$

The first product of acid hydrolysis is therefore the mandelonitrile glucoside obtained by Fischer; and this can be prepared in such manner. It was further shown that the action of yeast extract on amygdalin was due not to maltase but to the presence of a hitherto unknown enzyme appropriately termed amygdalase. This is more stable towards heat than maltase, and can be obtained almost free from maltase by preparing the extract at an elevated temperature.

The fact that an enzyme distinct from maltase effects the hydrolysis of amygdalin is clear proof that the glucoside does not contain maltose. Additional confirmation of this is afforded by the fact that the rate of hydrolysis of amygdalin either by amygdalase or by emulsin (ter Meulen) is not affected by the presence of maltose. This last sugar should have slowed the reaction had it been a constituent of the glucoside.

When amygdalin is hydrolysed by emulsin it is not possible at any stage of the reaction to detect the presence of a diglucose. In reality, under the influence of emulsin prepared from an aqueous

[1] According to the existing nomenclature *l*-mandelic acid forms *d*-mandelonitrile.

extract of almonds, two actions are going on at the same time, viz. hydrolysis at the centre Y, forming mandelonitrile glucoside and glucose, and, more slowly, hydrolysis of the mandelonitrile glucoside at X, forming benzaldehyde cyanohydrin and glucose. By interrupting the hydrolysis at the proper point it is possible to isolate the mandelonitrile glucoside. Such experiments prove that almond extract contains amygdalase in addition to the emulsin proper, which hydrolyses β-glucosides. Amygdalase is entirely without action on β-glucosides.

The second enzyme in emulsin has been found in the leaves of many plants where it occurs without amygdalase. Since it was first found in the leaves of the common cherry laurel it has been named prunase and the mandelonitrile glucoside on which it acts is termed prunasin.

"Emulsin" thus contains two enzymes, amygdalase and prunase, which act in turn on amygdalin. It is a remarkable fact that prunase is unable to act until the molecule has first been simplified by the action of amygdalase: this is taken as proof that the second molecule of glucose in some way shields the prunasin part of the molecule from attack by prunase. This explains the many unsuccessful attempts to obtain the disaccharide from amygdalin by means of plant enzymes.

This protective influence does not appear to apply, however, in the case of the enzymes present in the intestinal juice of the snail which, according to Giaja, are able to hydrolyse amygdalin in the first place to benzaldehyde cyanohydrin and a disaccharide, the latter subsequently undergoing further hydrolysis. The new carbohydrate is stated not to reduce Fehling's solution, that is, it is a disaccharide of the trehalose type. It has not been further investigated.

The amygdalin molecule is exceptional in containing several centres, marked X, Y, Z in the formula—

$$\underset{Z}{NC}.CHPh.\underset{X}{O}.C_6H_{10}O_4.\underset{Y}{O}.C_6H_{11}O_5,$$

totally different in their chemical nature, which are attackable by hydrolytic agents; its behaviour is, therefore, of the very greatest interest.

Amygdalin yields the same products (glucose, benzaldehyde and hydrocyanic acid) when treated with emulsin as when heated with dilute hydrochloric acid. In each instance the primary formation of *d*-mandelonitrile glucoside indicates that the biose junction Y is the first point to be attacked. The course of hydrolysis by concentrated acids is altogether different (Walker and Krieble). Concentrated

hydrochloric acid hydrolyses it to amygdalinic acid and ammonia in the first place at centre Z; subsequently, the amygdalinic acid breaks down at junction Y to *l*-mandelic acid glucoside and glucose so that junction X is the last point to be attacked. Concentrated sulphuric acid has very little tendency to attack the nitrile group at Z, the primary action being to eliminate *d*-mandelonitrile. The biose junction Y is the point most susceptible of attack by sulphuric acid at all concentrations. Sulphuric acid decomposes benzaldehyde cyanohydrin (junction Z) only with extreme difficulty.

In addition to *d*-mandelonitrile glucoside two other glucosides having the same composition are known. These are: prulaurasin, first described in the amorphous state under the name laurocerasin, and since obtained crystalline from the cherry laurel by Hérissey; and sambunigrin, separated by Bourquelot and Hérissey from the leaves of the common elder (*Sambucus niger*). These substances are both mandelonitrile glucosides; their properties are set out in the following table:—

TABLE XIV.

	M.p	$[\alpha]_D$.
Prunasin = dextro mandelonitrile glucoside[1] . . .	147°-150°	− 26·9°
Prulaurasin = racemic mandelonitrile glucoside . . .	120°-122°	− 52·7°
Sambunigrin = laevo mandelonitrile glucoside . . .	151°-152°	− 76·3°

Dunstan and Henry suggested that the differences between these lay in the nature of the sugar residue. This can hardly be the case, as they are all three attacked by emulsin, and therefore derivatives of β-glucose.

Prulaurasin is, in fact, a racemic mixture of the two stereoisomeric *d*- and *l*-mandelonitrile β-glucosides, and is analogous to isoamygdalin, the racemic form of amygdalin, which was first prepared by the action of alkali on amygdalin by Walker and subsequently studied by Dakin; it yields inactive mandelic acid when hydrolysed by acids; indeed, prulaurasin is obtained by acting on isoamygdalin with yeast extract—amygdalase (Hérissey). *Sambunigrin* is the β-glucoside of *l*-mandelonitrile glucoside, and derived from a still unknown isomeride of amygdalin. Prulaurasin is obtained from either of the other two isomerides, when their aqueous solutions are rendered slightly alkaline.

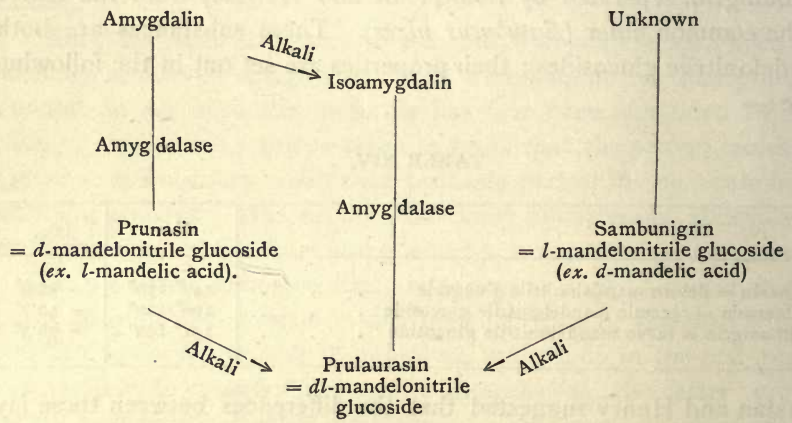
The true relationship of these glucosides was first established by

[1] According to the existing nomenclature *l*-mandelic acid forms *d*-mandelonitrile.

Caldwell and Courtauld, and their conclusions have been entirely confirmed by Bourquelot and Hérissey. More recently amygdonitrile glucoside has been discovered as a natural product, so that all three isomerides must play some part in plant economy. Hérissey found it in the young branches of *Cerasus Padus ;* Power and Moore have obtained it from wild cherry bark (*Prunus serotina*). It has been named prunasin.

The kernels of the cherry laurel contain as much as 4 per cent. of amygdalin : this plant, like most others, stores a more elaborate product in its seeds than is present in the leaves.

The inter-relationship of these compounds is indicated in the accompanying scheme. Possibly the unknown isomeride of amygdalin will also be found in the plant :—



The synthesis of the mandelonitrile glucosides has been successfully carried out by Fischer and Bergmann, who obtained the acetylated glucoside of ethyl mandelate in racemic form by treatment of the synthetic ester with acetobromoglucose and silver oxide in the conventional manner. A methyl alcoholic solution of ammonia transformed the ester into the corresponding glucosidic amide, and this was resolved into its pure optically-active forms by fractional crystallisation. The individual forms were converted by the action of phosphorus oxychloride into the tetra-acetyl derivatives of *d*- and *l*-mandelonitrile glucosides, which were, of course, identical with the tetra-acetates of prunasin and sambunigrin respectively.

On removal of the acetyl groups by hydrolysis racemisation sets in, and the product was found to be *r*-mandelonitrile glucoside, or prulaurasin. The synthetic racemic glucoside was resolved by

fractional crystallisation into *d*-mandelonitrile glucoside[1] (prunasin) and *l*-mandelonitrile glucoside (sambunigrin).

Cyanophoric Glucosides.

Hydrocyanic acid has frequently been isolated from plants, but it is only quite recently that its formation has been ascribed invariably to the decomposition of a glucoside. Besides amygdalin and the isomeric mandelonitrile glucosides a number of other glucosides have been isolated, which yield hydrogen cyanide when hydrolysed; they are conveniently grouped together under the term cyanophoric glucosides. Although rare compared with the occurrence of saponin in plants the distribution of hydrogen cyanide is proving much wider than was at one time imagined; its production has been observed in many plants of economic importance. A useful list of plants which yield prussic acid has been compiled by Greshoff. Some of the cyanophoric glucosides may be briefly mentioned:—

Dhurrin, first isolated by Dunstan and Henry from the leaves and stems of the great millet, is a *para*-hydroxymandelonitrile glucoside, and therefore closely related to the three mandelonitrile glucosides just described. Like them it is hydrolysed by emulsin.

Gynocardin, isolated by Power from the oleaginous seeds of *Gynocardia odorata*, yields prussic acid, glucose and an unknown substance, $C_6H_8O_4$, on hydrolysis. It is accompanied in the seeds by an enzyme, gynocardase, which also decomposes amygdalin.

Linamarin or *Phaseolunatin*, $C_6H_{11}O_5 . O . CMe_2 . CN$, was first isolated by Jorissen and Hairs from young flax plants and subsequently by Dunstan and Henry from *Phaseolus lunatus*. The latter authors consider it to be acetonecyanohydrin-*α*-glucoside, but it has since been shown to be a derivative of *β*-glucose. Hydrogen cyanide and acetone have been obtained from a number of plants on hydrolysis and possibly linamarin is widely distributed. It is the glucoside in the seeds of the rubber tree, *Hevea brasiliensis*. The glucoside is accompanied in plants by a specific enzyme linase which has been fully investigated by Armstrong and Eyre. *Phaseolus lunatus* contains two enzymes—an emulsin which, however, according to Dunstan, is without action on phaseolunatin and an enzyme of the maltase type which hydrolyses both phaseolunatin and amygdalin, forming mandelonitrile glucoside in the latter case. It is perhaps identical with the amygdalase described by Caldwell and Courtauld.

[1] Fischer uses the inverse notation, deriving the glucosides from *d*- and *l*-mandelic acids and not from their nitriles.

Linamarin has been synthesised by Fischer and Anger from β-acetobromo-glucose thus confirming its structure as a glucoside. The acetobromo-glucose condenses with ethylhydroxyisobutyrate to ethyl-tetracetyl-glucoside-α-hydroxybutyrate—

$$(OAc)_4 . C_6H_7O_5 . O . CMe_2 . CO_2Et,$$

which is extremely slowly hydrolysed by emulsin. Ammonia converts it into α-hydroxybutyramideglucoside—

$$C_6H_{11}O_5 . O . CMe_2 . CO . NH_2,$$

whilst on treatment with phosphorylchloride it yields tetracetyl linamarin.

Lotusin, discovered by Dunstan and Henry in *Lotus arabicus*, is of interest for two reasons. Like amygdalin it gives rise to two molecules of glucose on hydrolysis and therefore probably contains a disaccharide. The other products of hydrolysis are prussic acid and lotoflavin—an isomeride of fisetin. In the alkaline hydrolysis one of the glucose residues is obtained as heptagluconic acid, indicating that the cyanogen radicle is associated with the sugar residue. Lotusin is not hydrolysed by almond emulsin but it is resolved by an enzyme (lotase) which accompanies it, but as this also decomposes amygdalin and salicin it probably contains emulsin.

Vicianin has been found only in the seeds of a wild vetch, *Vicia angustifolia*. It is decomposed by an enzyme (vicianase) present in certain vetches into hydrogen cyanide, benzaldehyde and a disaccharide, $C_{11}H_{20}O_{10}$, vicianose, which is hydrolysed further by the emulsin of almonds into glucose and *l*-arabinose (Bertrand). Accordingly, vicianin represents amygdalin in which one molecule of glucose is replaced by arabinose.

A common grass *Tridens flavens* contains a considerable amount of hydrogen cyanide, the maximum quantity being present in the inflorescence tops. The quantity diminishes from August onwards, being nil in October.

Saponins.

The saponins are a numerous, widely distributed class of glucosides found in a great variety of plants; they are known to be present in more than four hundred plants belonging to about fifty different orders, and of these about fifty species have been studied and the saponins isolated.

Their most characteristic properties are the production of a soapy foam on mixing with water, and their toxicity, especially to cold-

blooded animals such as frogs and fishes; these were recognised as characteristics of the plants containing them in very early times, for example, by the Greeks.

All the saponins have many characteristics in common. Physically they are white or cream-coloured powders, in most cases colloidal and only dialysable with great difficulty, although recently several crystalline members of the group have been discovered. To the latter class belong several of the digitonin glucosides, parillin, sarsasaponin and cyclamin.

They are soluble in water, giving clear solutions which froth strongly on agitation, form emulsions with oils or resins, prevent the deposition of finely divided precipitates, and occlude electrolytes and also many soluble dye-stuffs. The saponin of soapwort (*Saponaria officinalis*), for example, in its colloidal form gives a blue adsorption compound with iodine, although the crystalline constituent does not (Barger and Field). In general the saponins are insoluble in ether, benzene, chloroform, and cold ethyl alcohol, but freely soluble in hot alcohol.

They possess a very bitter, acrid taste, and the dust of the powdered saponins is very irritating and sternutatory. As already mentioned, they are strong poisons to fish, the action here being of a chemical nature; on the other hand, they possess hæmolytic action of a more physical type, occluding the red corpuscles of the blood. The more poisonous saponins are referred to as sapotoxins.

The saponins may be broadly divided, from a chemical standpoint, into neutral and acid saponins. Formerly they were classified according to their formulæ, the earliest members which were studied forming an homologous series of the general formula, $C_nH_{2n-8}O_{10}$ (sometimes termed, after its discoverer, Kobert's series). Subsequently other saponins were found to belong to a similar series, $C_nH_{2n-10}O_{18}$, whilst more recently other glucosides, the properties of which entitle them to be classified as saponins, have been isolated and do not fall in either homologous series.

On hydrolysis the saponins yield a variety of sugars (frequently several molecules of carbohydrate), and physiologically active substances termed sapogenins; the latter have not as a rule been thoroughly examined, but are often compounds of a polyhydroxylactone nature. The sugars found to exist in combination with the sapogenins vary, glucose, galactose, and arabinose being the more common, whilst more rarely other pentoses and fructose are obtained.

All saponins form poisonous additive compounds with cholesterol.

Many sapogenins, such as those from guaiacum, saponaria, and digitonin give terpene oils when distilled in hydrogen with zinc dust (van der Haar).

The saponins are isolated from the root, leaves, seed, etc., of the plants by extraction with water and precipitation with neutral or basic lead acetate respectively, according as the saponin is acid or neutral. The precipitate is decomposed, and the solution evaporated, the residue being extracted with chloroform and precipitated by ether.

Some of the more interesting saponins will now be briefly discussed.

Saporubrin, $(C_{18}H_{28}O_{10})_4$, is a sapotoxin found in the root of the soapwort, *Saponaria officinalis;* on hydrolysis it gives a series of products, shedding one molecule of sugar at a time, until finally the sapogenin, $C_{14}H_{22}O_2$, is obtained.

Levant sapotoxin, $(C_{17}H_{26}O_{10}, H_2O)_2$, is very similar to saporubrin and occurs in the roots of *Gypsophila arrostii* or *G. paniculata;* it hydrolyses to four molecules of sugar (glucose and galactose) and a sapogenin, $C_{10}H_{16}O_2$. When gypsophila saponin is heated with dilute sulphuric acid a compound prosapogenin is obtained, which when heated with 2 per cent. sulphuric acid under pressure gives a monobasic ketonic acid, $C_{24}H_{34}O_5$, also a sapogenin (Rosenthaler and Ström).

Sarsaparilla glucosides.—Sarsaparilla, the dried root of *smilax* species, contains a mixture of saponins, amongst which are:—

Parillin, $C_{26}H_{44}O_{10}$, which hydrolyses to two sugars and parigenin, $C_{28}H_{46}O_4$, a phytosterolin, $C_{33}H_{56}O_6$, which gives glucose and a sitosterol on hydrolysis; sarsasaponin, $C_{44}H_{76}O_{20}$, $7H_2O$, crystals hydrolysing to three molecules of glucose and sarsasapogenin, $C_{26}H_{41}O_2(OH)$, and smilacin or smilasaponin (von Schulz), which is not a homogeneous substance, according to Power and Salway, who have most recently studied the sarsaparilla group.

Quillaic acid, $C_{19}H_{30}O_{10}$, and *Quillaia sapotoxin*, $C_{17}H_{26}O_{10}$, are respectively non-poisonous and poisonous constituents of the glucosides present in the bark of *Quellaja saponaria.* They are amorphous.

Agrostemma sapotoxin, $(C_{17}H_{26}O_{10})_2$, is a yellowish-white, highly poisonous amorphous glucoside found in the corncockle (*Lychnis* or *Agrostemma githago*); it is hydrolysed to four molecules of sugar and a sapogenin, $C_{10}H_{16}O_2$.

Digitonin and *Digitosaponin*, the saponin glucosides of the foxglove (*Digitalis purpurea*) have already been mentioned with the other digitalis glucosides.

The *hederins* or saponins of the ivy (*Hedera helix*) have been investigated by van der Haar and classified provisionally as α- and β-hederins, crystalline saponins insoluble in water, γ-hederin, amorphous glucosides insoluble in water, and Δ-hederin, the saponins soluble in water. At present only the α-hederin has been identified as an individual; it is a crystalline substance, $C_{42}H_{66}O_{11}$, hydrolysing to arabinose, rhamnose, and α-hederogenin, $C_{31}H_{50}O_4$, a dihydroxylic lactone.

The *polysciasaponins* occurring in *Polyscias nodosa* have been studied by the same worker, who has separated them by fractional precipitation into at least two individual members, α-polysciasaponin, $C_{22}H_{36}O_{10}$, and Δ-polysciasaponin, $C_{25}H_{42}O_{10}$. Both are white amorphous powders, which hydrolyse to one molecule each of arabinose, glucose, and a sapogenin, $C_{26}H_{44}O_4$, a saturated lactone containing neither hydroxyl, methoxyl, nor ethoxyl groups.

Other members of the group which may be cited are:—

Caulosaponin, $C_{54}H_{88}O_{17}$, and *Caulophyllosaponin*, $C_{66}H_{104}O_{17}$, two crystalline saponins found by Power and Salway in *Caulophyllum thalictroides*; *Jegosaponin*, $C_{75}H_{80}O_{25}$, from *Styrax japonica*, in which it occurs as a crystalline calcium salt: it gives on hydrolysis glucose, glucuronic and tiglic acids and a mixture of two sapogenins, $C_{33}H_{52}O_6$ and $C_{33}H_{52}O_7$; and two saponins, $C_{36}H_{56}O_{20}$ and $C_{37}H_{58}O_{20}$, from *Yucca angustifolia* and *Y. radiosa* respectively.

CHAPTER VIII.

THE SYNTHETIC GLUCOSIDES.

SEVERAL of the natural glucosides have been prepared synthetically, and by similar methods the corresponding glucosides of a variety of substances can be obtained. The starting-point for the synthesis of the natural glucosides was the crude acetochloro glucose prepared by Colley (1870) by the action of acetyl chloride on glucose. Michael (1879) coupled this with the potassium salt of phenols, preparing in this manner phenyl glucoside, helicin, salicin, and methylarbutin; Drouin by the same method obtained the glucosides of thymol and *a*-naphthol. Fischer in 1893 obtained the alkyl glucosides from acetochloro glucose, but they are more easily prepared as described in Chapter I.

Following the discovery of the crystalline *a*- and *β*-acetochloro glucoses attempts were made to extend and improve Michael's synthetical method, but were only successful in the case of the *β*-compound. As already mentioned, the *a*-acetochloro glucose in presence of alkali undergoes isomeric rearrangement to the *β*-acetochloro glucose, and accordingly *β*-glucosides result instead of *a*-glucosides.

Most of the glucosides synthesised have been prepared from the non-saccharide constituent and acetobromoglucose in presence of silver oxide. Fischer's most recent directions for the production of *β*-acetobromoglucose have been given previously on p. 27. Other synthetic glucosides have been derived from triacetylbromoglucosamine by Irvine and his co-workers, whilst a third and important group of syntheses is that effected, notably by Bourquelot, by means of enzymes.

The experimental methods available make it possible to synthesise almost any desired glucoside, especially since Fischer has shown how it is possible to obtain synthetic glucosides of the *a* series. In consequence, a variety of materials become available for the more exact study of the selective action of enzymes and the physiological activity of substances in combination with sugar.

The influence exercised by the non-sugar group on the stability of the glucoside and on its optical properties can also be studied.

The idea that glucosides are uniformly more reactive physiologically than the parent substances has not been well maintained.

The synthetic purine glucosides, also prepared by Fischer, may prove of medicinal as well as of scientific interest; they were obtained by the action of compounds of the type of β-acetobromoglucose upon the silver derivatives of the purines; glucosides, galactosides, and rhamnosides of adenine, guanine, hypoxanthine, theobromine and theophylline having thus been made. By condensation with phosphoric acid these compounds would be expected to produce synthetic nucleotides, and this was in fact attained when theophylline glucoside was treated with phosphorous oxychloride in pyridine solution, the product being a hydrated theophyllineglucosidophosphoric acid:—

$$[\underset{\text{Purine residue.}}{C_7H_7O_2N_4} - \underset{\text{Glucose residue.}}{C_6H_9O_5} - \underset{\text{Phosphoric acid residue.}}{HPO_2}], 2H_2O$$

The method of production of the purine glucosides has been patented by Bayer and Co.

Another synthetic nitrogenous glucoside is that of morphine (Mannich).

Interesting β-glucosides obtained by this method are those of menthol and borneol; they represent the first synthetical terpene glucosides, and are closely allied to the terpene glucuronic acid compounds. Similar glucosides include those of geraniol and cyclohexanol (Fischer and Helferich), and of citronellol, camphene, dihydrocarveol, fenchyl alcohol, terpineol, *cis*-terpene, sabinol, and santenol (Hämäläinen).

The β-glucosides of cetyl alcohol, glycollic acid, glycol (Fischer), menthol maltoside (E. and H. Fischer), and benzyl galactoside (Unna) have also been synthesised.

Salway has synthesised the similar ceryl and myricyl glucosides, and those of sitosterol and cholesterol; a contemporaneous investigation by Power and Salway showed that a number of natural compounds previously assumed to be phytosterols were really glucosides (phytosterolins). Amongst these were ipuranol, from olive bark (*Ipomœa purpurea*), etc., citrullol, found in colocynth and *Euonymus atropurpureum*, bryanol in bryony root, and cluytianol from *Taraxacum*.

Mauthner has synthesised glucovanillic acid, gluco-*p*-hydroxybenzoic acid, and other phenolcarboxylic acid glucosides, employing their methyl esters in the condensation with acetobromoglucose. He

has also prepared some of the gluco-hydroxy aceto- and benzo-phenones, the *p*-hydroxyacetophenone derivative being found naturally in pine needles, and known as picein (Tanret).

The glucosides of phloroglucinol, resorcinol, and 2 . 4 . 6-tribromophenol were obtained by shaking an ethereal solution of acetobromoglucose with the alkaline solution of the phenols; the first mentioned is identical with the glucoside obtained from phloridzin by Gremer and Seuffert and is capable of inducing diabetes (Fischer and Strauss).

Glucosides with long side-chains have been prepared by Bargellini by condensation of helicin and similar glucosides with different hydroxyketones, for example, with *p*-hydroxy acetophenone, when the compound—

$$C_6H_{11}O_5 . O . C_6H_4 . CH : CH . CO . C_6H_4 . OH$$

is formed.

These glucosides are stated not to be hydrolysed by acids and also not to be resolved by emulsin and therefore to belong to the *a* series. Since helicin is a *β*-glucoside this conclusion cannot be accepted and it is more probable that the long side-chain profoundly alters the properties of the glucoside.

Acetobromoglucose interacts with the silver salts of organic acids (Karrer) to form glucosides which are really acetylated glucose salts of the acids. The acetyl radicles could not be eliminated without at the same time removing the organic acid group so that the influence of the glucose molecule on the physiological activity of the acids employed could not be observed.

Irvine and Hynd have prepared *a*-aminohelicin and *a*-aminosalicin by condensing salicylic aldehyde and saligenin with triacetylbromoglucosamine in presence of morphine, morphine glucosamine appearing as a by-product. An aminomethylglucoside, different from that obtained by the action of ammonia on bromomethyl glucoside (Fischer and Zach), was prepared by the same workers from triacetylbromoglucosamine and methyl alcohol.

A new modification of the glucoside synthesis consists in warming acetobromoglucose with phenol in the presence of quinoline. During this process a rearrangement takes place and a mixture of *a*- and *β*-phenol glucosides is formed, which are separated by crystallisation from carbon tetrachloride. The *a*-phenol glucoside behaves normally in that it is hydrolysed by maltase but not by emulsin; acids, however, hydrolyse it nearly twice as quickly as the *β*-isomeride (see p. 131).

The method has been extended to menthol, and *a*- and *β*-menthylglucosides so obtained. The former is very sparingly soluble in water

and easily isolated, as much as 50 per cent. being obtained from β-acetobromoglucose. It is thus the easiest synthetic cyclic-α-glucoside to prepare and will be of interest for physiological studies. The α- and β-menthylglucosides behave normally towards maltase and emulsin and in this case the β-isomeride is somewhat the more rapidly hydrolysed by acids.

This synthesis of α-glucosides is of the utmost importance, as hitherto it has been impossible to obtain them owing to the fact that α-acetochloroglucose gave rise to β-compounds. The synthetic α-glucosides will allow of the further study of the influence of the arrangement of the groups on optical properties, resistance to hydrolysis by acids, etc.

It is of interest, further, that quinoline effects the rearrangement of the groups on carbon 1, whereas in the case of the transformation of gluconic into mannonic acid the groups attached to carbon 2 are affected.

Great interest attaches to the synthesis of the glucosides containing hydrogen cyanide. As described in the previous chapter Fischer has synthesised the natural glucosides derived from *d*- and *l*-mandelic acid and also linamarin, and the method will doubtless be extended to give synthetic material for the study of the many interesting chemical and physiological problems presented by these glucosides.

Synthetic Sulphur Glucosides.

Ethylthiomercaptal—

$$CH_2(OH).(CH.OH)_4.CH{<}^{SEt}_{SEt'}$$

when treated with one molecule of mercuric chloride loses one mercaptan residue only and ethylthio-glucoside is formed:—

$$CH_2(OH).\overset{\overset{\qquad O \qquad}{\frown}}{CH}.(CH.OH)_2.CH(OH).\underset{\underset{\qquad}{\smile}}{CH}.SEt$$

This crystallises in silky needles, m.p. 153°, $[\alpha]_D$ + 120·8°. It tastes bitter, does not reduce Fehling's solution, and is hydrolysed by acids but stable towards alkalis. No indication is given whether a second isomeride is formed at the same time. When excess of mercuric chloride is employed the mercaptal is reconverted into glucose.

By the interaction of β-acetobromoglucose and the potassium salt of thiophenol, β-thiophenol glucoside, $C_6H_5S.C_6H_{11}O_5$, has been obtained. This is not hydrolysed by emulsin and is very resistant towards hydrolysis by dilute acids. Analogous compounds have been

made by Schneider and co-workers from the silver salts of thiourethanes, the products having the general formula—

$$R.N:C(OC_2H_5).S.C_6H_{11}O_5$$

The products are amorphous and the acetyl free glucosides very easily undergo further hydrolysis into urethanes, $R.NH.CO_2Et$, and thioglucose which is readily isolated in the form of its silver salt. Decomposition also takes place in another way to form thiourethanes and glucose. This latter decomposition is met with in the case of the natural mustard oil glucosides, and phenylthiourethaneglucoside occupies an intermediate position between these and the aliphatic thiourethanes.

Myrosin is without influence on the synthetic thioglucosides.

Enzyme Synthesis of Glucosides.

The subject of the synthesis of glucosides by means of enzymes belongs properly to the monograph on enzyme action, and therefore only certain limited aspects of the question will be considered here.

Whereas in dilute aqueous solution the hydrolysis of say β-methylglucoside by emulsin is complete, hydrolysis is retarded by the presence of increasing amounts of methyl alcohol, until in presence of a certain proportion of alcohol the enzyme is able to synthesise glucoside from glucose and the alcohol. Definite proof that in this simple case the same glucoside is synthesised as is hydrolysed is afforded by its isolation in a pure state. Hence the reaction—

$$\text{glucoside} + \text{water} \rightleftarrows \text{glucose} + \text{methylalcohol}$$

is reversible and Bourquelot, to whom the development of this subject is primarily due, has proved that the ordinary physico-chemical laws governing such reversible reactions apply here also. For example, the rates of hydrolysis and synthesis are the same and the same equilibrium is reached from both directions. The temperature limits of these reactions and the proportions of the various alcohols which can be used without destroying the activity of the enzyme have been determined. Yeast extract, i.e. maltase, effects the synthesis of α-methylglucoside or galactoside. Emulsin and also Kephir lactase are able to produce β-methylgalactoside.

The reaction has also been extended successfully to other alcohols, the enzyme being allowed to act on sugars dissolved in alcohols containing varying amounts of water or acetone. In this way crystalline glycol, glycerol, geranyl and cinnamyl-β-glucosides and alkyl and benzyl galactosides have been obtained by means of emulsin: some of these had not been induced to crystallise when made by other

methods. These synthetic glucosides are hydrolysed by emulsin. Latterly many other alcohol glucosides have been prepared in like manner, for example, those of the terpene alcohols: these, it will be remembered, are transformed into glucuronates in the animal system.

The work more particularly of Armstrong has shown that enzymes are very active hydrolytically even when quite insoluble in the medium employed. Thus finely ground leaf material, prepared by protracted autolysis and frequent washing with water, and therefore divested of all soluble matter, was very active towards salicin and other glucosides.

Similarly emulsin is capable of synthesising and hydrolysing β-glucosides in a neutral liquid such as acetone in which it is completely insoluble.

The view that synthesis and hydrolysis are effected by different enzymes, though not overlooked by earlier workers, has been brought into prominence by the experimental work of Rosenthaler. Emulsin in presence of hydrogen cyanide and benzaldehyde brings about the formation of optically active benzaldehyde cyanohydrin, a substance which it also hydrolyses. Saturation of the enzyme solution with magnesium sulphate or half-saturation with ammonium sulphate produces a precipitate which is soluble in water. The filtrate has no synthetic activity, but is able to effect hydrolysis as before; the precipitate possesses synthetic activity and some hydrolytic activity. It is considered by Rosenthaler that emulsin consists of two distinct enzymes, one promoting synthesis, the other causing hydrolysis of benzaldehyde cyanohydrin.

It must not be overlooked that enzymes as we know them are mixtures of several, often closely related, enzymes. Subtle differences exist between different preparations, as is shown by Krieble's observation that an emulsin which produced *l*-mandelonitrile from amygdalin, two years later produced the *d* variety. It is suggested that two synthetic enzymes are present in emulsin and acting on benzaldehyde and hydrocyanic acid to produce the *d*- and *l*-nitrile respectively, the latter enzyme being less stable.

Krieble also states that the emulsin from sweet almonds produces the *l*-nitrile and that from bitter almonds the *d*-nitrile. An enzyme converting benzaldehyde and hydrogen cyanide into *d*-mandelonitrile is present in the leaves and bark of *Prunus serotina*, which it will be remembered contains prunasin, the glucoside of this nitrile. From the leaves of the elder, which contains sambunigrin, no optically active compound was obtained. Emulsin, as just indicated, forms the racemic nitrile and also an excess of one of the optically active forms.

An interesting synthesis of salicin and other glucosides is that studied by Ciamician and Ravenna. When plants—well-grown maize plants were chosen—are inoculated with glucosides or their aromatic products of hydrolysis a reversible change takes place resulting in a chemical equilibrium. Salicin is in part hydrolysed, saligenin in part transformed into salicin, the final ratio in the full-grown plant of combined to free saligenin being 1 : 2. On taking a large number of plants it was possible to isolate the salicin synthesised in this manner. Confirmation of this work appears desirable.

It is suggested by Ciamician and Ravenna that the absorption by both adult and germinating plants of certain aromatic compounds leads to the formation of the corresponding glucosides in the plants. Thus maize and beans when treated with weak saligenin solutions form salicin.

CHAPTER IX.

THE FUNCTION OF CARBOHYDRATES AND GLUCOSIDES IN PLANTS.

CARBOHYDRATES are of fundamental importance in plants: quite apart from the process of assimilation in which starch is formed, the carbohydrates and more particularly their glucosidic derivatives are now recognised as playing an all-essential part in other physiological processes. Sufficient space is not available in the present monograph for more than a brief indication of some of the more developed branches of this field of inquiry in which work is now being done in many directions.

The last few years have witnessed great progress in the novel interpretation of the function of glucosides as a means of keeping dormant substances of great importance in the metabolism of the plant until the precise moment at which they are required. The so-called respiratory and anthocyanin pigments are derived from glucosides, likewise many perfumes. Similarly a large class of substances, which are capable of acting as hormones and giving a very delicate but directed stimulus to plant metabolism, are constituents of glucosides.

Since any particular glucoside is only hydrolysed by its specific enzyme, the supply of these materials for whatever purpose they are required is regulated by a very sensitive control. The glucoside-enzyme systems are to be regarded as constituting a controlling mechanism for plant metabolism.

Significance of Glucosides.

Opinions are divided as to the real significance of glucosides in plant economy. Probably they are of use to the plant in a variety of ways, and no one explanation will cover the functions of all the members of the group.

In most, if not in all cases, the glucosides are accompanied by appropriate enzymes which are able to hydrolyse the glucoside. Enzyme and glucoside do not exist in the same cells as normally there is no decomposition. They are brought together should the cellular structure be damaged and in some instances during germination.

In the cherry-laurel, according to Guignard, "emulsin" exists in the endodermis; in the almond, it is found in the axis of the embyro in the pericycle which lies immediately under the endodermis, and in the cotyledons in both the endodermis and the pericycle. Bourquelot, who prepared both glucoside (gaultherin) and enzyme from the stems of *Monotropa*, showed they are not present in the same cells.

The earliest investigations of this nature are due to Marshall Ward. The fruits of the Persian berry (*Rhamnus infectorius*) contain a glucoside known as xanthorhamnin, which, when hydrolysed, yields rhamnetin and the two sugars rhamnose and galactose. Marshall Ward and Dunlop showed that the seeds contain an enzyme, termed rhamnase, capable of hydrolysing the glucoside; this is confined to the raphe of the seed, which is composed of parenchymatous cells containing a brilliant oily-looking colourless substance. When the pulp or an extract of the pericarp of the fruit is digested with an extract of the seeds a copious yellow precipitate of rhamnetin is formed.

In very many cases glucosides function as reserve materials, and when required they are hydrolysed by the accompanying enzyme and pass into circulation.

It would appear that the glucoside stored in the seed is often of a more complex character than that present in the leaves of the same plant, containing more than one sugar or two molecules of the same sugar in its molecule, whereas the leaf glucoside is a simple one. A special enzyme is required to hydrolyse it which is present only in the seed and absent from the leaf.

Thus the seeds of Prunus species contain amygdalin together with the enzymes, amygdalase and prunase, required for its complete hydrolysis; the leaves contain mandelonitrile glucosides and prunase but no amygdalase. Complex glucosides are present in the seeds of other plants, as indicated in the previous chapter.

Anæsthetics such as chloroform or ether are well known to have a remarkable action on plants in stimulating growth. Of the deepest significance in this connection is Guignard's observation that exposure of living plants to the action of anæsthetics brings about interaction between the glucoside and the corresponding ferment. Mustard oil is formed from the leaves of certain cruciferæ, hydrogen cyanide from laurel leaves and other cyanophoric plants, when submitted to the action of chloroform. The same phenomenon is brought about by exposure to extreme cold.

The investigations of H. E. and E. F. Armstrong have shown that a variety of substances, having the property in common that they

have but little affinity for water, are able to penetrate the walls of certain plant cells. As a consequence alterations in equilibrium are set up within the cell, and changes are induced which involve alteration of the concentration and the liberation of hydrolytic enzymes.

The general name hormone has been applied to substances which are active in this manner: it has been shown that the group includes not only carbon dioxide but materials such as hydrogen cyanide, hydrocarbons, alcohols, phenols, ethers, esters, aldehydes, mustard oils, etc., all of which are normal products of hydrolysis of the plant glucosides. The hormones include most of the substances which Overton, Löb, Czapek and others have classed as solvents of lipoids.

The result of the liberation of enzymes within the cell will be hydrolysis of complex carbohydrates, glucosides, proteins, etc., and the materials so formed will be active in still further stimulating change. If unchecked, change will proceed until autolysis is complete: in practice the intervention of oxydases is made manifest by the appearance of brown and other pigments.

It will be seen that the plant cell carries its own hormones or activators in an inactive form bound up as glucosides. If for any reason during the twenty-four hour period a little of the glucoside becomes hydrolysed, the hormone will be liberated and a very delicate stimulus given to the cell to begin down-grade changes such as normally take place at night.

Glucosides and Animal Nutrition.

The recognition of the potent effect of the constituents of glucosides in acting as stimuli and starters of active metabolism may be of importance in studying the nutrition of animals. It is well known that the herbage of one pasture may have the power of fattening an animal whereas similar grass on an adjoining field, though equally readily consumed by the animal, fails to bring it into condition for the market.

This is especially the case in Romney Marsh, where one field will fatten six or eight or more sheep to the acre whereas an adjoining field will do little more than keep the sheep in a growing condition. Hall and Russell, who investigated this difference in 1912, found that the floral type in the two fields was constant but that a leafy habit of growth obtained in the fattening field and a stemmy habit in the poorer fields. The ordinary methods of chemical analysis failed to reveal any difference either in the herbage or the soils. Since this date much evidence has accumulated in favour of the importance of quality as

well as of quantity in animal feeding and the subject is one of the greatest importance to agriculturalists.

Subtle differences between the grasses of these two fields have hitherto defied detection, but it is not impossible that the presence or absence of certain glucosides or similar constituents may have some bearing on the difference.

H. E. and E. F. Armstrong have made observations on the behaviour of *Lotus corniculatus* collected during several years both over Great Britain and a greater part of Europe. Whereas *L. corniculatus* usually contains a cyanogenetic glucoside and the corresponding enzyme, it is established that a botanically indistinguishable form exists from which the glucoside is absent.

Lotus uliginosus, which some botanists regard as a distinct species, is free both from the glucoside and the correlated enzyme: it grows, as a rule, in damp situations and is distinguished by its rank growth and coarse tubular stem. The normal form of *L. corniculatus* contains both glucoside and enzyme; a widely distributed second form is rich in enzyme but lacks the glucoside, and a third rare form lacks both glucoside and enzyme.

Lotus ranks rather as a weed than as a fodder plant and is a minor constituent of most pastures but it is of great interest that white clover, *Trifolium repens*, shows similar differences. Two varieties are recognised by seedsmen—the cultivated and wild—of which the latter is often said to be the more valuable. The wild variety contains a cyanogenetic glucoside and the correlated enzyme, whereas the cultivated lacks glucoside and has very little enzyme.

A further example is afforded by linseed cake, which is considered superior to all other cakes as a food in bringing cattle into condition. Owing to the presence of the cyanogenetic glucoside linamarin in the unripe seed a small quantity of hydrogen cyanide is usually to be found in linseed cake.

Glucosides are likely to play a very important part as "test materials" in the solution of this and many problems of plant chemistry. Their non-sugar constituents can frequently be detected with great accuracy and delicacy and even localised *in situ* in the tissue and they also can be estimated quantitatively. In this respect the glucosides which yield hydrogen cyanide on hydrolysis are of particular value, more especially as many hundreds of qualitative tests can be made in relatively short time.

In testing for cyanide it is most convenient to make use of stout glass tubes, about $3\frac{1}{2}$ inches long and $\frac{1}{2}$ inch wide, provided with good

corks. The leaf material having been pushed into the tube, two or three drops of chloroform or toluene are added and a slip of moist picrate paper is inserted; the tube is then corked up. It is conveniently incubated in a waistcoat breast-pocket or in the trousers pocket. When cyanide is present the paper reddens perceptibly within half an hour, as a rule; to make certain, the test should be prolonged over 24 hours. To prepare the picrate paper, slips of filter paper about $\frac{3}{8}$ inch wide are dipped into a solution of 5 grm. picric acid and 50 grms. sodium carbonate in 1 litre of water; after draining the paper, it is hung from a pin to dry until it is only just perceptibly moist; it is then cut up into $\frac{3}{4}$-inch lengths and stored in a closed tube. It is well to keep a piece of the paper in each of the stock of tubes carried, so as to make sure that hydrogen cyanide has not been stored up in the cork.

Glucosides may also serve as a method of putting harmful waste products out of action: thus phenolic residues are rendered soluble by combination with glucose and are transferred osmotically to other portions of the plant.

Bunge has pointed out that very many of the non-sugar constituents of glucosides are antiseptic and therefore bactericidal in character. In the seeds of plants the reserve stores of food-stuffs form an excellent medium for the development of micro-organisms which would rapidly spread but for the protective action of the glucoside. In the almond, directly the seed is penetrated, the amygdalin is hydrolysed and all bacterial action prevented. The universal presence of glucosides in the bark of plants may be similarly explained: they ensure an antiseptic treatment of all wounds in the integument.

Easily decomposable substances, such as many acids or aldehydes, are protected against oxidation by being transformed into glucosides, just as, in the animal organism, similar substances are converted into paired glucuronic acid derivatives.

Glucosides possessing a bitter taste or having poisonous properties serve to protect such important organisms as the seeds or fruits of plants against animals. In some instances the plant is only poisonous at certain stages of its growth. Thus an Egyptian plant, *Lotus Arabicus*, is poisonous in the early stages, but becomes a useful forage when allowed to mature: it contains the glucoside lotusin, which yields hydrogen cyanide when hydrolysed.

Glucosides containing acetonecyanohydrin are regarded by Treub as primary material for protein synthesis. Guignard, working with

phaseolunatin, has obtained no evidence that hydrocyanic acid is liberated during germination of Phaseolus beans.

The amount of glucoside present varies considerably in different species of the same plant, and varies also according to the time of year. It also differs in the male and female plant of the same species. Unfortunately, the material at present available for the discussion of this question is very scanty. Jowett and Potter, who investigated the bark from thirty-three samples of willow and poplar, found considerable variation in the occurrence of salicin. In April the bark from the female tree contained about three times as much salicin as that from the male; three months later the conditions were reversed. It is suggested that salicin acts as a reserve material; it is stored away in the winter for use in the coming spring, when it is hydrolysed by the accompanying ferment, both saligenin and glucose being used by the plant. The plant is enabled to store in the form of a glucoside a material which it can neither tolerate in quantity nor produce at short notice when required. Owing to their special functions the reserve is drawn upon to an unequal extent by the male and female trees. Taxicatin, the glucoside of the leaves and young shoots of the yew (*Taxus baccata*), occurs in greatest quantity in the plant during the autumn and winter; apparently it is utilised in the spring when the young shoots begin to assimilate. The cyanophoric glucoside in the leaves of *Sambucus nigra*, according to Guignard, seems to fulfil a different function, as its amount diminishes only slightly with age, and at the end of the vegetative period the glucoside does not migrate to the stems but remains in the leaves till they drop off.

The variations in the composition of the root of the gentian during a year's growth have been studied by Bridel. The gentian root contains a glucoside, gentiopicrin, and the carbohydrates glucose, fructose, sucrose, and gentianose (p. 112), the last of which is hydrolysed by invertase. The amount of carbohydrate hydrolysed by invertase increases from a minimum (1·2 per cent.) early in June to a maximum (7·8 per cent.) in August and then remains constant. The amount of glucoside (2 per cent.) does not vary much; it increases a little in June and July. In May and June gentianose is largely replaced by gentiobiose. The sucrose increases from 1 per cent. in July to 4 per cent. or more in November: it is absent when growth commences in the spring.

According to Cavazza the amount of tannin in the leaves of forest trees reaches a maximum in September, whereas the amount in the

twigs shows maxima in July and December and varies inversely as that in the leaves.

A comparison of the amount of glucoside and enzyme in linseeds grown under conditions of drought and high temperature with those grown under damp and low temperature conditions has been made by Collins and Blair. Under the latter conditions the total amount of hydrogen cyanide produced falls 20 per cent. but the activity of the enzyme increases by a like amount. This is the general effect of growing linseed in this country, whereas seeds of oriental origin are rich in total hydrogen cyanide.

Respiration in Plants.

Carbohydrates and glucosides are concerned likewise in the phenomena of respiration in plants, during which oxygen is absorbed, carbon dioxide given off and the energy necessary for carrying out the life-work of the plant liberated. The process of oxidative decomposition of food substances is separable into two stages: in the first, alcohol and carbon dioxide are produced, as may be demonstrated by allowing pea seeds to germinate without the access of air. The anærobic process of carbohydrate decomposition, if not identical with, is very similar to the alcoholic fermentation of glucose by yeast.

The second stage in respiration comprises the ærobic oxidation of the alcohol produced in the first stage: this is effected, according to the present view, by the agency of the respiratory pigments which are themselves present originally as glucosides and liberated by hydrolysis. No doubt, salts of iron, manganese, etc., play some part in the oxidative changes, but their precise function is not yet understood.

Important light has been thrown on the function of the aromatic substances in plants and on the existence of enzymes, which act only on them, by the researches of Palladin. Following the line of thought originated by Reinke, who discovered substances in the plant which, under the influence of enzyme (oxydase) and air, gave coloured oxidation products, Palladin made a systematic search for these respiratory chromogens. He supposes them to be cyclic compounds bound to carbohydrates in the form of insoluble glucosides. Glucoside-splitting enzymes separate the cyclic compounds, which by the aid of the oxydases are then enabled to take up oxygen from the air to give it up again later under the influence of reducing substances. During life the chromogens normally remain colourless so long as there is a balance in the activities of the three types of enzyme concerned, but, on treatment with chloroform or other hormones, or after death due to cold

or injury, the inter-relationship of the enzymes is disturbed and the coloured oxidised chromogen becomes evident.

Prochromogen (i.e. glucoside) + water → chromogen + sugar.

Chromogen + oxygen → respiration pigments.

The soluble pigments of flowering plants—red, purple, and blue—which are termed collectively anthocyanin by botanists, are regarded similarly as oxidation products of chromogens of an aromatic nature, probably in many cases members of the flavone and xanthone groups (Wheldale): there is little doubt that these colourless chromogens are present in the living tissues as glucosides.

In the flavone glucosides one or more hydroxyl groups are replaced by sugar: hence, since the auxochrome group is replaced, the crude plant extracts are only pale yellow. On hydrolysis the sugar is split off and the colour deepens and a deposit of flavone is formed as the glucoside is more soluble than the pigment. Wheldale suggests that the reactions involved are in general terms as follows :—

glucoside + water $\rightleftarrows$ chromogen (flavone) + sugar.

chromogen + oxygen → anthocyanin.

The evidence in favour of this hypothesis is summarised by Wheldale in her monograph on the anthocyan pigments.

Everest, however, obtains anthocyanin by cautious reduction in the cold of the pigments in a number of pale yellow or white flowers. Under these conditions no anthocyanidin is produced and no oxidation after reduction is necessary for the production of the anthocyanin pigment.

Combes has found that red leaves of which the coloration is attributed to anthocyanin contain proportionately greater amounts of glucosides and sugars than green leaves of the same plant; Kraus has proved the same to hold for the aromatic constituents. The evidence as to the formation of anthocyanin has been summarised by Wheldale; it is regarded as due to the accumulation of glucosides. Sugar feeding increases both the amount of glucoside and of free aromatic chromogen.

The autumnal coloration of leaves is attributed (Overton, Tswett) to the same series of changes brought about by the slowing up of the metabolic processes of the plant by frost and other influences resulting in the disturbance of the enzyme balance. Tannins, for example, when set free from their glucoside form by the hydrolytic enzymes, yield pigments on oxidation (cf. p. 68).

The production of pigment may involve something more than the interaction of the aromatic chromogen with the oxydase. Chodat has

accumulated evidence showing that protein decomposition products, i.e. the amino acids or polypeptides, also take part in the reaction, and the precise shade of colour produced depends on the nature and quantity of these as well as on that of the aromatic compound derived from the glucoside.

The formation of the anthocyan pigments is of great interest from the point of view of genetics. The three groups of factors concerned are:—

(1) Actual formation of the pigment.
(2) Amount of pigment formed.
(3) Modification of the colour of the pigment.

For the formation of the pigment two factors, the chromogen and the oxidising agent or enzyme, are required. The work of Keeble and Armstrong has correlated the distribution of the oxydases with that of anthocyan. The amount of pigment formed may be controlled by an enzyme liberating more or less from the corresponding glucoside. The variation in the colour from red to blue or purple is regarded by Willstätter and others as determined by the nature of the accompanying substances in the cell sap, but this explanation is not in harmony with the biological facts. It is more likely that other substances are present, as for example, in centaurea, in which the purple, red, and blue types contain cyanin and the pink variety pelargonin.

Carbohydrates and the Enzyme Balance.

In dealing with carbohydrate metabolism in plants there is abundant evidence that a very delicate balance exists between the various enzymatic processes which take place simultaneously, leading, it may be, to the building up of starch or to the transference of a glucoside into anthocyanin.

It is obvious that the introduction from without of agencies which will affect this balance will have a more or less profound influence in altering the changes which take place.

One of the most delicate means of regulating the balance is that afforded by change of temperature. A rise or fall in the temperature does not influence all enzyme reactions alike—for example, some are retarded by cold much more than others.

A typical case is that afforded by the potato tuber during storage (Müller-Thurgan). Three changes take place simultaneously: starch is being transformed into sugar, sugar into starch, and also by the process of respiration into carbon dioxide. A decrease in the temperature hinders all three reactions but it has least effect on the

formation of sugar from starch. Accordingly, when the potato is stored at 0° sugar is formed till the amount increases to 3 per cent. At − 1° all enzyme action ceases. At + 3° there is still formation of sugar but the enzymes acting to destroy it tend to keep the amount down to 0·5 per cent. At + 6° the rate of formation of sugar from starch and that of the reverse change are equal; above this temperature the formation of starch predominates. In consequence no sugar is stored and any sugar previously present is destroyed.

The effect of a further rise in temperature on the enzyme balance has not been worked out in such detail but there is no doubt that the influence is equally profound. This conception of the regulation of metabolism affords an explanation of the sudden development of plant growth due to a warm day in spring when the rise in temperature favours synthetic changes; or of the injury caused to hot-house plants by exposing them to a temperature colder than that to which they are accustomed, whereby an abnormal preponderance of hydrolytic activity is favoured which, if unduly prolonged, may lead to the disintegration of the protoplasmic structure and death of the plant.

In the case of plants which are killed by frost it is supposed that as a result of the removal of the water as ice the concentration of the cell fluid becomes such that the soluble proteins are precipitated from solution. This salting out of the proteins is prevented by the presence of non-electrolytes such as sugar: Lidforss, to whom this explanation is due, has shown that the leaves of winter plants are free from starch but contain much sugar. The warm days of early spring bring about the regeneration of starch and partial disappearance of sugar; in consequence the cell is but ill protected against the effects of a subsequent frost.

The Ripening of Fleshy Fruits.

In the first stages after fertilisation the changes in the young fruit resemble those in the leaf: a variety of acids, tannins, and sometimes starch then accumulate, and ultimately, as the fruit becomes ripe, carbohydrates and fruit ethers or aromatic substances are formed and the bitter, acid, or astringent taste disappears together with the starch.

The interrelationship of the materials concerned and the enzymes which effect their transformation possesses numerous points of interest —the scope of the present work limits discussion here mainly to the carbohydrates. A distinction has been drawn between three types of fruit (Gerber) which in the preliminary stages are rich either in acids,

tannins, or starch: the subsequent changes differ somewhat in each type.

As a typical starchy fruit the banana may be considered. During ripening there is an evolution of carbon dioxide and a considerable conversion of starch into sugar. Thus Prinsen-Geerligs found during six days the amount of starch decreased from 31 to 9 per cent., the cane sugar rose from 0·8 to 13·6 per cent., and the invert sugar from 0·25 to 8·3 per cent. The presence of oxygen is necessary for ripening; in an atmosphere of nitrogen the starch remains intact.

A careful study of the enzymes present in extracts of bananas gathered at different stages of ripening has been made by Tallarico. The catalytic enzyme which decomposes hydrogen peroxide is very active in the green fruit but weakens as it ripens. Diastase is only active in the green fruit or at the beginning of ripening, it then disappears. Invertase is absent during the green stage, the amount very rapidly increases during ripening and then gradually disappears. A proteoclastic enzyme is evident during ripening and then likewise vanishes. Maltase is not present at any period.

During ripening the skin of the banana changes from green to yellow, deep brown, and finally black; the fruit is then fully ripe. This change is due to an oxydase acting on some aromatic substance liberated from a glucoside. The black colour is quickly produced when a yellow banana skin is disintegrated by mincing or when the entire skin is exposed to the vapour of some hormone. Under natural conditions the stimulus which leads to blackening is given from within the fruit by the liberation of the characteristic ester of the banana, which acts as a powerful hormone. In the case of most fruits, it would seem that the final appearance which is associated with ripeness is conditioned by stimulus from within rather than by any environmental influence.

Vinson has found that invertase is present in the date throughout the green stages but remains in an insoluble *endo* form: during ripening it becomes readily soluble, changing to the *ecto* form. The change coincides very closely in point of time with the conversion of the soluble tannins into an insoluble form. The unripe date contains much cane sugar; in the ripe fruit this is converted into invert sugar. Influences, such as have been considered under the name of hormones, which destroy the structure of the protoplasm, liberate the endo-enzyme, provided always that the dates have reached a certain stage of development.

The acids in fruits are chiefly malic, tartaric, and citric. Gerber

considers that during ripening they are in part converted into sugar and in part oxidised to carbon dioxide. Temperature has an important influence on the rate of oxidation. Experiments with fungi (*Sterigmatocytis*) have shown that whereas at 12° glucose is attacked preferentially to tartaric acid, at 20° the rate of attack is equal, at 37° the tartaric acid is least resistant. Malic acid is oxidised more easily than glucose at all temperatures: fruits containing it, such as apples, can ripen, therefore, in colder climates than those containing tartaric acid, like grapes. Citric acid is still more resistant to attack, and fruits such as oranges and lemons require warmer climates in order to ripen.

In apples, according to Kelhofer, the percentage of sugar is highest in the flesh, the acidity increases towards the centre, the tannin from the centre outwards. The distribution is the same in ripe as in unripe apples, but during ripening the amount of acid greatly diminishes.

In oranges (Scurti and Plato) citric and malic acids are present; during ripening the quantity at first increases but then becomes much smaller. Sucrose diminishes in amount, glucose and fructose increase.

During the ripening of sloes (Otto and Kooper) the amount of fructose increases whilst that of glucose decreases together with the acids and tannin: the loss is in part due to respiration. The same authors have studied the changes in medlars and quinces during ripening.

In the ripening of cereals the object is to store starch instead of converting it into sugar. The enzymes act synthetically and there is a gradual accumulation of carbohydrate within the endosperm tissue. The slowly matured, plump grains contain a higher proportion of starch than the small and rapidly ripened grains.

In the sweet potato, *Ipomœa batatas*, the conversion of starch into sugar is apparently connected with the cessation of the activity of the leaves, as until the stem is cut off or the tubers harvested very little sugar is formed. The transformation results first in the production of reducing sugars from starch which are then converted into sucrose. It is of interest that the change, although slower at low temperatures, ultimately goes much further, so that much more sugar is formed.

Products Derived from Carbohydrates in Plants.

Carbohydrates are the first products of synthesis in the plant, and the other products of plant activity must be expected in large measure to arise from them. For example, unripe seeds, nuts or fruits contain carbohydrates, which as ripening proceeds are transformed into fats and oils. This change is of the greatest interest, particularly in regard to the variety of fats found in nature and their great commercial importance. Two changes are involved: the alcoholic hydroxyl groups must be reduced and the short carbon chains of the carbohydrates must be condensed to form the long chains characteristic of the higher fatty acids. It will be remembered that these consist always of an even number of carbon atoms and are unbranched, suggesting that a decomposition product of glucose containing two carbon atoms is formed and takes part in the condensation, the reaction being repeated so that each successive acid has two more carbon atoms. Nef's work has shown that the glucose molecule may be disjointed at either the α- or β- or γ-carbon atoms: in the latter case lactic acid is formed, as happens when glucose is acted on by bacteria or by weak alkalis. The first product of fission is perhaps glycerose—

$$CH_2(OH) . CH(OH) . CHO$$

which by rearrangement becomes lactic acid—

$$CH_3 . CH(OH) . CO_2H$$

Both glucose and lactic acid can undergo butyric fermentation in which butyric acid, $CH_3 . CH_2 . CH_2 . CO_2H$, is formed.

Nencki suggested that lactic acid breaks down into acetaldehyde, hydrogen and carbon dioxide and that the aldehyde undergoes repeated aldol condensation followed by the same rearrangement as in the case of the formation of lactic acid from glycerose:—

$$CH_3 . CHO + CH_3 . CHO \rightarrow CH_3 . CH(OH) . CH_2 . CHO \rightarrow CH_3 . CH_2 . CH_2 . CO_2H$$

The reaction involved in the reduction of the β-alcoholic hydroxyl accompanying the oxidation of the aldehyde group is apparently a general one in carbohydrate metabolism. The further study of this fascinating subject belongs to the domain of fat chemistry, for which the monograph of Dr. Leathes should be consulted.

The aldehydes present in green leaves have been investigated by Curtius and Franzen who worked up 600 kilograms of the leaves of the hornbeam for this purpose. They identified formaldehyde, acetaldehyde, η-butylaldehyde, valeraldehyde, $\alpha\beta$-hexylene aldehyde and higher homologues. The hexylene aldehyde—

$$CH_3 . CH_2 . CH_2 . CH : CH . CHO$$

formed the greater part, and considerable quantities of acetaldehyde and butyric aldehyde were present, the other aldehydes being present only in small amounts.

It is suggested that this aldehyde is produced from glucose by the repetition of the following series of changes:—

$$\begin{array}{l} CH_2.(CH.OH)_4.CHO \\ | \\ OH \end{array} \quad \text{glucose,}$$

$$CH_2:C(OH).(CH.OH)_3.CHO \quad \text{water eliminated,}$$

$$CH_3.CO.(CH.OH)_3.CHO \quad \text{water added and eliminated,}$$

$$CH_3.CH(OH).(CH.OH)_3.CHO \quad \text{reduced,}$$

involving, in the first place, the formation of a methylpentose, and subsequently, on repetition of the reduction, of an ethyl tetrose which would have the same composition as digitoxose. Kiliani assigns a different structure to this sugar, but it requires reinvestigation from this point of view.

The constitution of the enzymes which act on the carbohydrates has long been a subject of speculation. According to H. E. and E. F. Armstrong the enzyme has the double function of retaining the hydrolyte in circuit while hydrolysis is being effected by an electrolyte formed from an active radicle present in the enzyme. The acceptor portion of the enzyme must be compatible with a grouping common to all the members of the series of glucosides which it hydrolyses, and it has been postulated as an amino glucose composing part of a large colloid molecule. Carbohydrates have been shown to be a component even of highly purified enzymes though it is not yet possible to carry the purification very far without destroying the activity of the enzyme.

Willstätter finds that the purest and most active peroxydase he could prepare from a very large quantity of horse radish consisted chiefly of a nitrogenous glucoside containing about 30 per cent. of a pentose and the equivalent proportion of a hexose with about 3 atoms of nitrogen. Peroxydase is not, of course, generally regarded as an enzyme in the same sense as the sugar-splitting compounds, and the method of purification would have inevitably destroyed the sucrolastic enzymes. It is none the less of considerable interest that a very active catalytic agent should be composed so largely of sugar molecules.

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